PRELIMINARY RISK ASSESSMENT: PHASE I

BENZIDINE, ITS CONGENERS, AND THEIR DERIVATIVE DYES AND PIGMENTS

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Foreword

Evaluations of chemical substances prepared by scientists in EPA's Office of Testing and Evaluation, Office of Pesticides and Toxic Substances (OPTS), to implement provisions in the Toxic Substances Control Act (TSCA), will be published periodically and made available to the public in the TSCA Chemical Assessment Series. Some of the volumes in the series are reports on single chemicals; others are compendiums of information received and evaluated by the Agency about many chemicals. (The anticipated frequency of publication will vary among titles: some will be published annually, some semiannually, and others quarterly.)

Because the chemical assessments published in this series often will reflect initial or intermediate steps in EPA's evaluation of a chemical under TSCA, the Agency welcomes the submission of additional information for or comments on its evaluations. Such submissions will be considered either at a subsequent step in the assessment of the subject chemical or in the decision not to proceed with further evaluation.

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Introduction

In the Office of Pesticides and Toxic Substances (OPTS), the assessment of chemical substances is sequential. A decision to perform a Preliminary Risk Assessment (Phase I assessment) of a chemical is based on a Chemical Hazard Information Profile (CHIP)* or its equivalent.

Following validation of the key information in the preliminary risk assessment, a detailed risk assessment (Risk Assessment in Support of Regulatory Decision Making, Phase II) is prepared if the Phase I disposition indicates that comprehensive assessment should continue.

The Preliminary Risk Assessment report is a summary and analysis of information on the known sources and effects of exposure to a given chemical, identifying the specific problems it is most likely to create. The report arrives at preliminary conclusions about which effects present significant hazards; whether existing or anticipated exposure levels may pose a threat to human health or the environment; whether the information available on a chemical is sufficient to complete all or part of the Preliminary Risk Assessment; and the key technical and policy issues that the information in the report has raised.

The structure of the Preliminary Risk Assessment report has evolved over time to include an executive summary, an introduction to the chemical, information about production and use, health and environmental effects, principal sources of exposure, and a preliminary analysis of risk. As a result of the evolution of the structure and content of the report, early Phase I reports do not share a common outline and have not gone through the same process of development as those now being prepared. However, in all Phase I reports, to the extent that it is possible to do so, potential sources of the chemical and its potential effects are ranked on the basis of relative risk. This ranking considers any differences in absorption route, frequency, or duration of exposure that may cause ranking by relative risk to differ from ranking by relative exposure level.

The literature search for a Preliminary Risk Assessment report is targeted to retrieve 95 percent of all published information on a given chemical for a 30- to 50-year period. This search should turn up all relevant published articles, reviews of the literature, research monographs, and monitoring and field study reports. The search does not include unpublished studies, particularly those in industry files. To obtain such information, a TSCA section 8(d) reporting requirement may be

*The Chemical Hazard Information Profile (CHIP) is a brief summary addressing potential for adverse health or environmental effects or significant exposure based primarily on secondary literature. For a more extensive discussion of the CHIP, see the Introduction in "Chemical Hazard Information Profiles (CHIPs), August 1976-August 1978," Volume 1, April 1980, EPA 560/11-80-011.

invoked. If essential use and production data are not available from the section 8(b) Inventory or any other internal Agency source, a section 8(a) reporting requirement may be invoked. However, when a substantial amount of data is available in the published literature, the literature search generally will suffice.

A Preliminary Risk Assessment report is accompanied by a disposition summary documenting the next course of action planned on the chemical. The three major possible dispositions are to keep the chemical in the assessment process, to suspend further assessment based on existing information, and to initiate information-gathering efforts (such as testing requirements under section 4 of TSCA) to obtain information critical to further assessment.

At this stage in the process the Preliminary Risk Assessment report is published in the TSCA Chemical Assessment Series, with a request for public comment on the report's accuracy and completeness. All comments received are available for inspection and copying in the OPTS reading room,* unless specifically claimed as confidential in accordance with applicable EPA rules and procedures (see 40 CFR Part 2 [41 FR 36902, Sept. 1, 1976]). Public comments received on a Phase I report are considered in the validation stage, which will be described subsequently, or in reconsidering a decision not to proceed to validation.

The second stage of the preliminary risk assessment is the validation of the Phase I assessment report and the preparation of a Control Options paper. The validation is an in-depth staff review of the adequacy of the key studies and the conclusions drawn about exposure and effects. To date, not all of the Phase I reports written in OPTS have been validated. The Control Options paper analyzes the feasibility of using various regulatory controls to reduce the most serious of the risks presented by a chemical.

Once a Preliminary Risk Assessment report has been validated, the report and its corresponding Control Options paper are submitted to the Toxic Substances Priority Committee (TSPC). The TSPC considers whether the chemical should proceed to initiation of rulemaking under TSCA or other authorities, based on the Committee's evaluation of the relative importance of the sources of environmental release and exposure, the severity and probability of potential effects, the feasibility of the proposed controls, and the mechanism to effect the needed controls.

Validated Preliminary Risk Assessment reports will be published if they differ substantially from the unvalidated reports.

*Room E447 at EPA Headquarters, 401 M Street SW, Washington, D.C. 20460

Executive Summary

Benzidine and its congeners, 3,3'-dichlorobenzidine, o-tolidine (3,3'-dimethylbenzidine), and dianisidine (3,3'-dimethoxybenzidine) constitute a family of similar synthetic aromatic compounds (see Figure 1).

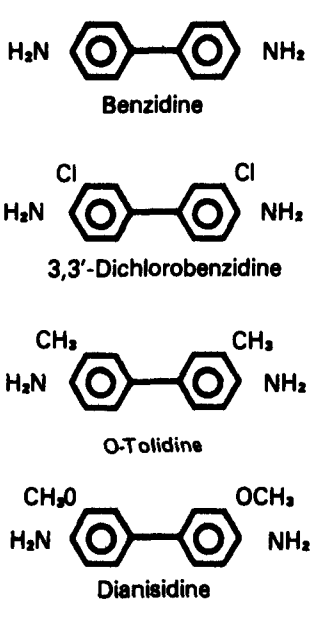


Figure 1. Chemical structures and solubility properties of benzidine and its congeners.

These compounds are important as precursors in the synthesis of 85 dyes and pigments that are commercially available in the United States*: namely 22 benzidine-based dyes, 34 dianisidine-based dyes, 22 o-tolidine-based dyes, 5 dichlorobenzidine-based pigments and 2 dianisidine-based pigments (see Table 1). These dyes and pigments are used to color textiles, rubber, plastic products, printing inks, paints, lacquers, leathers, and paper products.

*Although there are only 85 such dyes and pigments currently on the U.S. market, the technology exists for producing more than 450 dyes and pigments (listed in the Colour Index [1971]) that are based on benzidine or one of the three benzidine congeners.

Table 1. Dyes and Pigments Derived from Benzidine and Its Congeners Currently on the U.S. Market, 1979

Derived from benzidine:

Direct Black #4, 38
Direct Blue #2, 6
Direct Brown #1A, 2, 6, 31, 59, 74, 95, 154
Direct Green #1, 6, 8
Direct Orange 8
Direct Red #1, 28, 37
Direct Violet #1, 22
Acid Red 85

Derived from o-tolidine:

Acid Red #114, 167
Acid Black 209
Azoic Coupling Component 5
Azoic Yellow Composition #1, 2, 3
Azoic Orange Composition 3
Direct Blue #14, 25, 26
Direct Orange 6
Direct Red #2, 39
Direct Brown 230
Direct Yellow 95

(Dyes without C.I. generic name)
Diphenyl Green
Direct Fast Brown BCW-NB
Padazoic Yellow G
Padazoic Orange GR
Penetrating Black AM-NB
Sandolan Red N-3B

Derived from dianisidine:

Direct Black #94, 114, 118
Direct Blue #1, 8, 15, 22, 76, 80, 80(S),
90, 98, 100, 151, 160, 191(S),
218, 218/224 (S), 244,
269 (o-anisidine)
Direct Brown 200
Direct Yellow 68
Direct Violet 93
Azoic Blue Composition #2, 3, 6
Azoic Diazo Component 48
Azoic Coupling Component 3

Table 1. Dyes and Pigments Derived from Benzidine and Its Congeners Currently on the U.S. Market, 1979 (cont.)

(Dyes without C.I. generic names)
Atlantic Printing Black 2B P
Atlantic Resin Fast Blue ARL
Padazoic Brilliant Indigo 3B
Atlantic Printing Brown GGN
Atlantic Resin Fast Grey LVL
Superlitefast Rubine WLKS
Derived from dianisidine:
Pigment Orange 16
Pigment Blue 25
Derived from dichlorobenzidine:
Pigment Orange #13, 34
Pigment Yellow #12, 13, 14

Source: Information supplied by Dyes Environmental and Toxicology Organization, Inc. (DETO), and Dry Color Manufacturer's Association

Benzidine and its three congeners all have been shown to be mutagenic in the Ames Salmonella assay. Benzidine also is a demonstrated animal carcinogen and has been shown to cause bladder cancer in humans. Positive carcinogenicity studies (some of questionable validity by current standards) also have been reported for the three benzidine congeners.

Three benzidine-based dyes (Direct Blue 6, Direct Black 38, and Direct Brown 95) were reported to be carcinogenic in rats. These dyes, as well as Direct Red 28, have been shown to metabolize to benzidine in rhesus monkeys. Preliminary results suggest that Direct Red 28, and five additional benzidine-based dyes not previously tested, are also metabolized to benzidine in dogs.

The o-tolidine-based component of Trypan Blue is a proven teratogen and mutagen; the commercial dye, which is of mixed composition, is known to be a carcinogen. No studies investigating the carcinogenic potential of dianisidine-based dyes have been found in the available literature. Metabolism tests in dogs are currently under way to determine whether o-tolidine- and dianisidine-based dyes are metabolized to the parent compounds.

Pigments Yellow 12, 13, and 83 (dichlorobenzidine-based) and Pigment Yellow 16 (o-tolidine-based) are not carcinogenic in rats or mice. Moreover, metabolism tests performed in the same species have failed to demonstrate the release of free dichlorobenzidine from Pigments Yellow 12 and 13. Pigments Yellow 12 and 13 differ structurally from the above benzidine-based dyes for which there is positive evidence of carcinogenicity, accompanied by proof of metabolic breakdown to release the parent benzidine compound. In addition to a difference in solubility

(pigments are insoluble, whereas the dyes are water soluble), dichlorobenzidine is a benzidine derivative with chlorine substituents ortho to the amines; a keto-enol tautomeric group adjacent to the azo linkage in the pigments may shield this linkage from enzymatic cleavage. There currently is insufficient information, however, to draw firm conclusions about the mutagenic, teratogenic, or carcinogenic potential of other pigments derived from dichlorobenzidine or the other benzidine congeners. Tables 2 and 3 summarize the evidence regarding the carcinogenicity, teratogenicity, and mutagenicity of benzidine, its three congeners, and the dyes and pigments derived from them.

There is little published information on the environmental fate of benzidine, its congeners, and the dyes and pigments derived from them. Benzidine is thought to be oxidized readily in clay soils (with a half-life of less than 1 day) and air (a half-life of 1 day) but less readily in water (a half-life of 100 days, with organic peroxide ions). One field study suggests that benzidine-derived dyes and pigments (in waste waters from manufacturing plants) are reduced to the parent compound by hydrogen sulfide or sulfur dioxide in the receiving waters. Adequate measures exist for processing wastewaters to reduce the level of free benzidine to below 10 ppb, a level at which benzidine can be biodegraded by unacclimated activated sludge in subsequent sewage treatment works. However, the biological effects of the by-products of this process have not been assessed. Regardless of the potential biodegradation and restricted environmental release of benzidine, recent studies suggest that, if available, benzidine is bioaccumulated in certain fish. Dichlorobenzidine also has been shown to bioaccumulate (135-fold) in bluegill sunfish. The potential for bioaccumulation and possible contamination of the food chain by benzidine (and dichlorobenzidine) may be enhanced by the lack of restriction on release of dichlorobenzidine into the environment. Recent studies suggest that the latter compound is rapidly degraded in aqueous solutions (by natural sunlight), forming benzidine and 3-chlorobenzidine.

Direct exposures, both in terms of the number of people exposed and in exposure levels, to benzidine, its congeners, and their derivative dyes and pigments occur primarily in occupational settings. Since 1976, occupational exposure to benzidine and benzidine-based dyes has been greatly reduced as a result of a general reduction in the level of production and of the imposition by the Environmental Protection Agency (EPA) of standards for controlling benzidine levels in effluents from manufacturing plants and dyeing operations. Currently there is only one major manufacturer of benzidine-based dyes in the United States; only a few U.S. manufacturers still produce dyes and pigments based on the three benzidine congeners. The reduction in U.S. production, however, has been accompanied by an increase in the importation of benzidine-based dyes, in which the level of free benzidine is not controlled. In some imported dyes the level of free benzidine has been reported to be as high as 500 ppm (25 times higher than normally found in U.S. dyes). Thus, workers in plants using imported benzidine-based dyes may constitute a new high-risk group.

The general public is exposed to benzidine, its congeners, and their derivative dyes and pigments mainly in some paper, textile, leather, and other substrates. Once applied to the substrate the dyes or pigments are generally regarded as fast--i.e., they do not readily leach from the material when used (e.g., when laundered) according to label directions.

Table 2. Summary Information on Carcinogenic, Mutagenic, and Teratogenic Effects of Benzidine and Its Congeners

Compound	Carcinogen		Mutagen		Teratogen	
	Human	Animal	Strong	Weak	Human	Animal
Benzidine	Yes (Bladder)	Yes (oral, 4 species)	+		n.r.*	n.r.*
Dichlorobenzidine	n.r.*	Yes (oral, 3 species)	+		n.r.*	n.r.*
o-Tolidine	n.r.*	Yes (subcutaneous, 2 species)		+	n.r.*	n.r.*
Dianisidine	n.r.*	Yes (oral, 1 species)		+	n.r.*	n.r.*

* n.r.--Not recorded in the available literature.

Table 3. Summary Information on Carcinogenic, Mutagenic, and Teratogenic Effects and Metabolism of Benzidine-Related Dyes and Pigments

Base compound	Carcinogen	Mutagen	Teratogen	Metabolized to base compound
Benzidine	Direct Blue 6 Direct Black 38 Direct Brown 95	Direct Violet 1 Direct Red 28	n.r.*	Direct Black 4 Direct Black 38 Direct Blue 2 Direct Blue 6 Direct Brown 95 Direct Green 1 Direct Orange 8 Direct Red 28
Dichlorobenzidine	Negative results, Pigments Yellow 12, 13, 83	n.r.*	n.r.*	Negative results, Pigments Yellow 12, 13
o-Tolidine	Negative results, Pigment Yellow 16	Trypan Blue†	Trypan Blue	n.r.*
Dianisidine	n.r.*	n.r.*	n.r.*	n.r.*

*n.r.--Not recorded in the available literature

†Active ingredient is pure o-tolidine-based component of dye mixture

The possible extent of dye leaching under normal use conditions, however, has not been determined for any of the dyes or pigments considered in this report.

Other sources of exposure affect relatively small segments of the general population but may be of greater concern. These include the packaged dyes for home use (which may contain benzidine or benzidine-congener derivative dyes) and those artist-craftsman products that contain benzidine-congener derivative dyes and pigments--i.e., spray paints, enamels, and lacquers.

Although additional information on exposure, release, and environmental fate is needed to complete a detailed risk assessment on benzidine, its congeners, and their derivative dyes and pigments, several potential risks have been identified through a preliminary analysis of the exposure and hazards associated with these compounds. The risks are (a) to workers exposed to imported benzidine-based dyes that contain high concentrations of free benzidine; (b) to workers using domestically produced benzidine-based dyes (there are no occupational exposure standards for either benzidine or its derivative dyes); (c) to the general population, which may result from exposure to benzidine-based dyes in a variety of products including home dyes and textiles; and (d) to the environment from the release of dichlorobenzidine.

An assessment of the health risks that may be attributed to o-tolidine, dianisidine, and their derivative dyes and pigments depends on the validity of the carcinogenicity studies on the congeners and the potential for metabolic conversion of the dyes/pigments to the parent compound(s).

Disposition

Benzidine is one of 15 chemicals identified for assessment by Douglas Costle, Administrator, U.S. Environmental Protection Agency. It is now in the validation stage of preliminary risk assessment.

I. PRODUCTION AND USES

A. Production Methods

1. Benzidine and Congeners

Benzidine is produced from nitrobenzene in two steps. First, nitrobenzene in alkaline solution is treated with a mild reducing agent to form what is mainly hydrazobenzene and lesser amounts of azobenzene and azoxybenzene. Subsequent treatment of hydrazobenzene with mineral acid results in the formation of benzidine by the well-known benzidine rearrangement (Lurie 1964). The reaction scheme for the synthesis of benzidine is shown in Figure 2.

At present, benzidine is produced domestically solely as a precursor of azo dyes, by a single dye manufacturer, Fabricolor, using hydrazobenzene as the starting material (Boeniger 1979). The synthesis of benzidine congeners (Figure 3) is similar to benzidine synthesis, with nitrobenzene derivatives being used as the starting materials.

2. Dyes and Pigments

The distinction between the terms "dye" and "pigment" as stated by the dye industry (and as used in this report) is that dyes are soluble and pigments are insoluble in the medium in which they are used. Where solubility is not an important consideration, the word "dye" is sometimes used to refer to dyes and pigments collectively, as in Lurie (1964).

Production of dyes and pigments from benzidine and its congeners proceeds via tetrazotization to form the tetrazonium salt, which is followed by coupling of the tetrazonium compound with a relative compound (e.g., aromatic hydroxy compounds or arylamines) to form a colored product (Lurie 1964). Tetrazotization is accomplished by reacting benzidine or a congener with nitrous acid (sodium nitrite in hydrochloric acid) in a water solution at 0-5°C. This produces the coupling agent, a tetrazonium hydrochloride (Figure 4).

The final step in the production of a dye solution is the coupling of the tetrazonium salt with a phenol, aromatic amine, or other reactive compound. The coupled products have azo groups that are linked to sp^2-hybridized carbon atoms; hence, they are azo dyes. A typical azo dye made from benzidine (Congo Red, the first azo dye produced from benzidine) is shown in Figure 5.

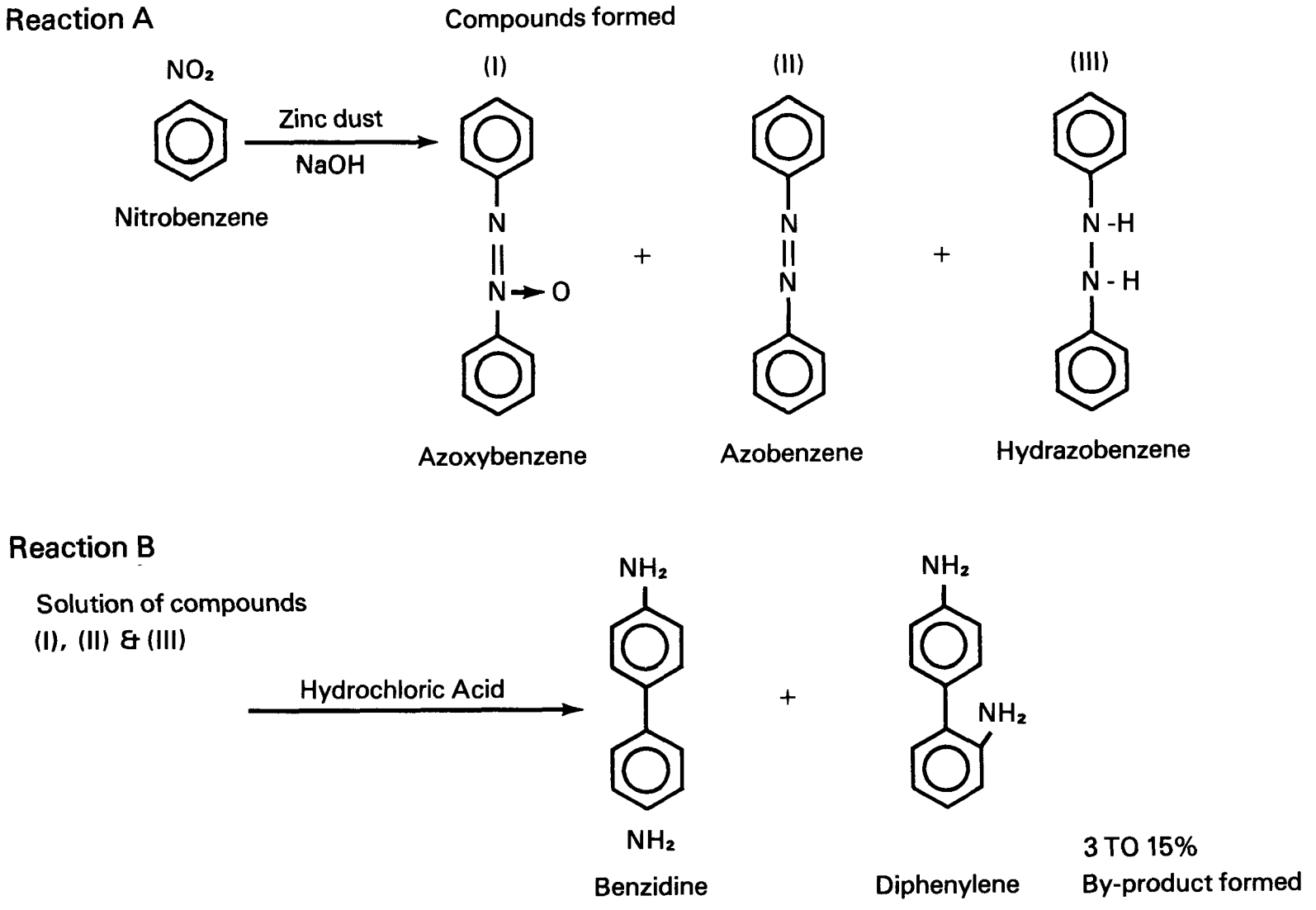


Figure 2. Synthesis of benzidine.

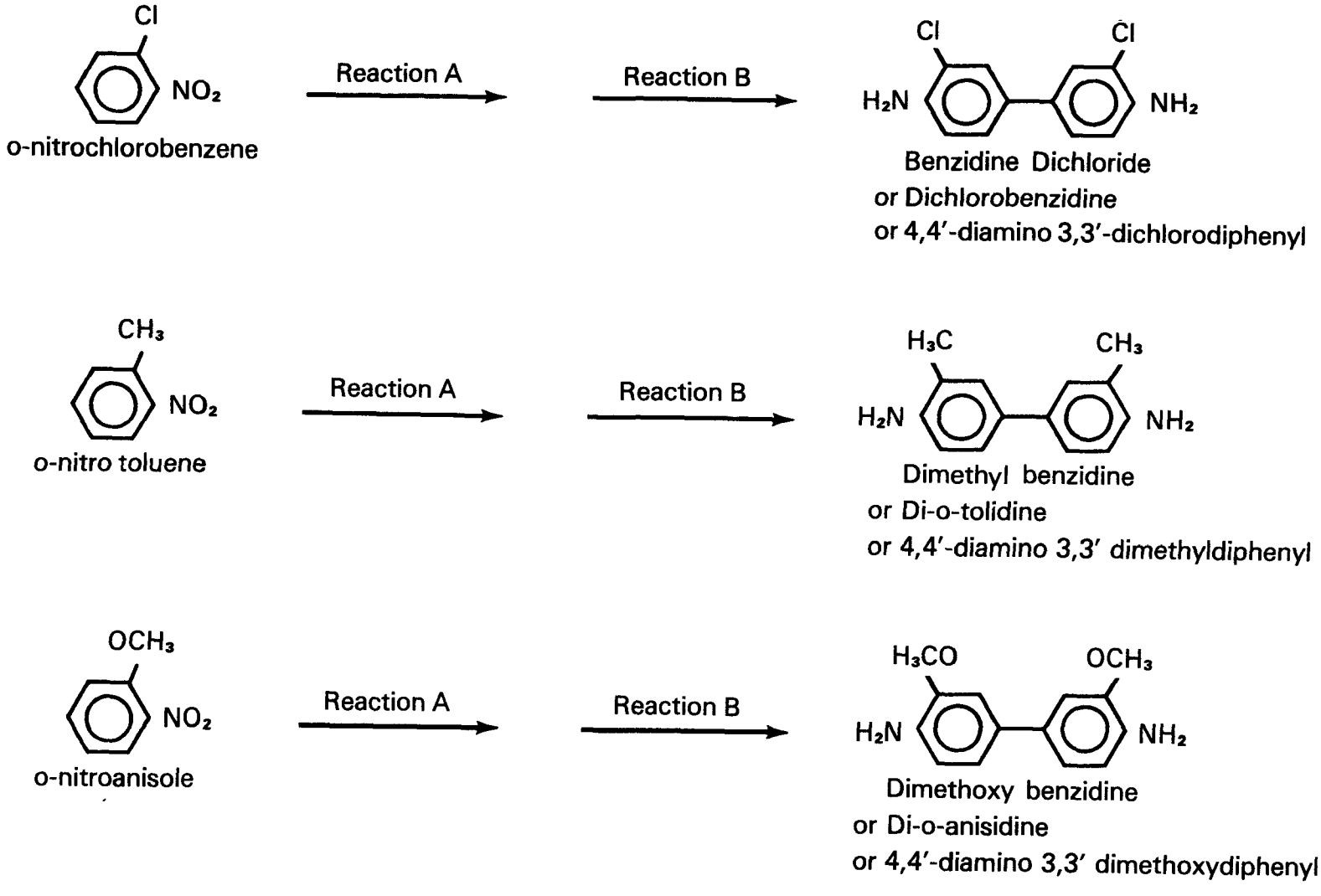


Figure 3. Synthesis of benzidine congeners.

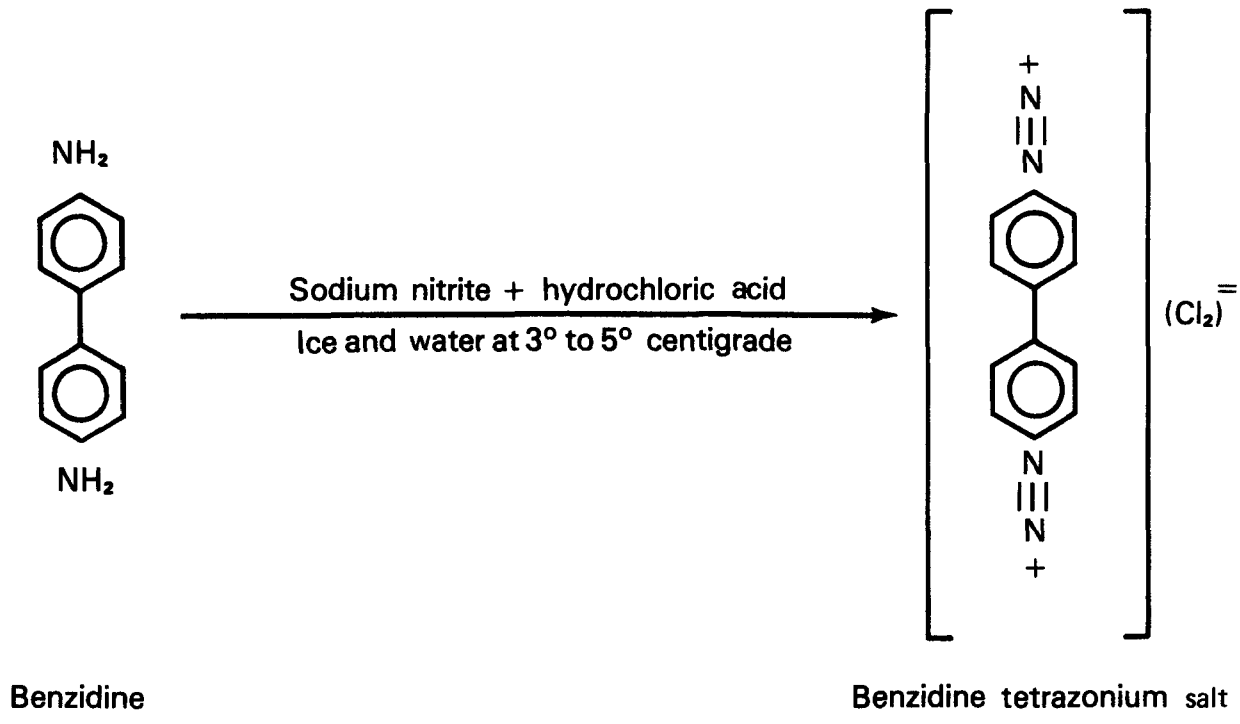


Figure 4. Tetrazotization of Benzidine.

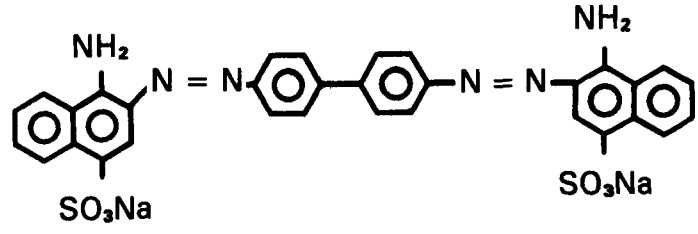


Figure 5. Structure of Congo Red (Direct Red 28).

In the coupling reaction the first diazo group of the tetrazonium salt of benzidine or a congener couples readily to give the diazomonoazo compound. The activity of the second diazonium group is diminished so that formation of unsymmetrical disazo dyes also can be accomplished (Lurie 1964). By proper choice of coupling agents, the resulting diazo compound can be diazotized or used itself as a coupling component with another diazonium compound to give rise to bisazo, trisazo, tetrakisazo, or polyazo dyes (Lurie 1964). A wide variety of dyes spanning the color spectrum is made possible by (1) a large number of possible coupling agents, (2) the choice of symmetrical or unsymmetrical coupling, and (3) the possibility of forming monoazo or polyazo derivatives. Table 4 shows the coupling agents and their arrangements in some typical benzidine-based azo dyes.

Table 4. Azo Dyes Derived from Coupling Benzidine

Reaction (◄— Indicates diazo coupling)	Color
Salicylic acid ◄— Benzidine —► Salicylic acid	Orange
Naphthionic acid ◄— Benzidine —► Naphthionic acid	Congo Red
H-acid ◄— Benzidine —► H-acid	Blue
Salicylic acid ◄— Benzidine —► Gamma acid	Brown
Salicylic acid ◄— Benzidine —► H-acid ◄► p-Nitroaniline	Green
m-Phenyldiamine ◄— Benzidine —► H-acid ◄► Aniline	Black

Source: Adapted from Lurie (1964)

B. Major Uses

By far the most important and largest use of benzidine in the United States is the production of the 22 benzidine dyes currently in commercial production. These dyes are among the class known as direct dyes because they can be applied directly to cellulosic substrates (e.g., cotton and paper) without use of mordant. Relatively small amounts of benzidine also have been used in analytical reagents to determine the presence of certain inorganic ions (Welcher 1947); in thin-layer chromatography (Adamovic 1966); in analytical reactions to determine the presence of a number of organic compounds (IARC 1972a); and in security printing, because it reacts with ink erasers (IARC 1972a).

Dichlorobenzidine can be used for coupling to produce the 95 tetrazo dyes listed in the Colour Index (1971); however, of these, only five pigments are currently produced in the United States. Dyes and pigments produced from dichlorobenzidine can be used for coloring plastic resins, lacquers, rubbers, printing inks, metal finishes (Martens 1968), textile and wallpaper prints (Colour Index 1971), interior grade "lead-free" finishes (paints and toy enamels), and floor coverings (Colour Index 1957). A few hundred metric tons of dichlorobenzidine also were used in 1969 as a curing agent for liquid castable polyurethane elastomers (Woolrich and Rye 1969).

o-Tolidine is used domestically to produce 22 azo dyes. These dyes are used for coloring products similar to those listed for benzidine and dichlorobenzidine.

Dianisidine is used domestically to produce 36 azo dyes. These are used for coloring leather, paper, plastics, rubber, and textiles. A small quantity of dianisidine also has been used to manufacture 3,3'-dimethoxy-4,4'-diphenylene diisocyanate, an ingredient in isocyanate adhesives and a component of polyurethane elastomers (Woolrich and Rye 1969).

C. Production and Import Volumes

1. Benzidine and Congeners

With the recognition of the potential health hazards of benzidine, its manufacture and use have come under regulation in the United States and in some foreign countries (Ferber 1978). By 1975, a number of U.S. producers had ceased manufacturing benzidine, and by 1978 its use was reported to be diminishing rapidly (Ferber 1978). Although a complete compilation of information on production and import volumes of benzidine and its congeners could not be derived from available sources, the fragmentary data compiled in Table 5 support the conclusion that benzidine production in the United States has been greatly reduced since 1974. The general lack of information on imports precludes an assessment of a trend in benzidine importation; there were no imports reported by the International Trade Commission in 1978. Production of dianisidine in 1978 was greatly reduced from 1967, the only previous year for which production figures are available; imports show no obvious long-term trend. Production of dichlorobenzidine rose threefold between 1962 and 1977, while imports fluctuated greatly; in general, however, imports seem to have decreased since 1970. Since 1974, production of o-tolidine has remained stable; imports have fluctuated since 1971.

Table 5. Production and Imports of Benzidine and Its Congeners

	Chemical Production (lb)	Imports (lb)
	Benzidine*	
1978	Small amount for research only (a)	None (a)
1977		9,500 (a)
1976	1,100,000 (b)	None (a)
1975	1,540,000 (b)	No data
1974	1,327,000 (c)	No data
	3,3'-Dichlorobenzidine	
1978	Estimated at several million (a)	261,705 (e)
1977	5,500,000 estimated in (a)	None (a)
1976	4,500,000 estimated in (a)	2,002
1975	3,000,000 estimated in (a)	None (a)
1972	6,424,000 (b)	No data
1970	3,656,000 (d)	979,812 (d)
1968	2,940,000 (d)	928,786 (d)
1962	1,702,000 (c)	No data

Table 5. Production and Imports of Benzidine and Its Congeners (cont.)

	Chemical Production (lb)	Imports (lb)
	Dianisidine	
1978	"Very small quantities once or twice a year" (a)	554,012 (e)
1977	†	428,007 (a)
1976	No data	751,784 (a)
1975	†	400,780 (a)
1972	No data	272,800 (f)
1967	367,400 (f)	No data
1960	360,000 (c)	No data
	o-Tolidine	
1978	200,000 est. (g)	330,547 (e)
1977	200,000 (a)	353,281 (a)
1976	200,000 (a)	472,794 (a)
1975	†	312,351 (a)
1974	220,000 estimated in (b)	600,000 (b)
1971	No data	97,680 (f)
1962	243,000 (c)	No data

Source: Figures include base compounds and salts, where reported separately. Data sources: (a) Powell et al. (1979, pp. 4-2, 4-3), (b) Ferber (1978), (c) Lurie (1964), (d) Gerarde and Gerarde (1974), (e) USITC (1979), (f) IARC (1972b), and (g) NIOSH (1978b).

*Production volume may not include benzidine produced in situ for dye manufacture.

†The U.S. International Trade Commission does not give production statistics when there are fewer than three producers or when one producer is dominant.

2. Dyes and Pigments

The available production and import data for specific dyes and pigments derived from benzidine and its congeners for the period 1975-1978 are presented in Table 6. Dyes and pigments listed are those for which figures were reported for more than one year. Most notable is the drop in production of benzidine-based dyes; no significant trends can be discerned for dyes and pigments based on o-tolidine, dianisidine, and dichlorobenzidine.

Table 7 summarizes the available total sales and import data for dyes and pigments based on benzidine and its congeners. Over the 4-year period, a significant drop in U.S. sales of benzidine-based dyes can be seen, as

Table 6. Production and Import Data for Dyes Based on Benzidine and Its Congeners

Dye		1975	1976	1977	1978
Based on benzidine:		(lb)	(lb)	(lb)	(lb)
Direct Black 38	Produced	2,168,000	3,759,000	*	None
	Imported	*	70,753	49,525	170,442
Direct Blue 2	Produced	*	771,000	*	None
	Imported	11,023	38,478	*	30,755
Direct Brown 2	Produced	125,000	188,000	*	None
	Imported	2,205	18,739	2,205	None
Direct Brown 31	Produced	73,000	47,000	*	None
	Imported	*	*	*	None
Direct Brown 95	Produced	346,000	595,000	*	None
	Imported	11,023	8,205	15,962	5,512
Direct Green 1	Produced	132,000	169	*	None
	Imported	747	*	*	None
Direct Green 6	Produced	*	*	*	None
	Imported	250	*	*	4,659
Direct Red 1	Produced	132,000	62,000	*	None
	Imported	*	*	4,409	None
Acid Red 85	Produced	67,000	72,000	*	None
	Imported	500	2,190	*	None

Table 6. Production and Import Data for Dyes Based on Benzidine and Its Congeners (cont.)

Dye		1975	1976	1977	1978
		(lb)	(lb)	(lb)	(lb)
Based on o-tolidine:					
Acid Red 114	Produced	*	*	240,000	None
	Imported	*	*	24,200	2,310
Based on dianisidine:					
Direct Black 91	Produced	*	*	*	*
	Imported	*	3,527	3,527	*
Direct Blue 1	Produced	*	236,000	186,000	*
	Imported	*	*	*	*
Direct Blue 15	Produced	*	*	530,000	*
	Imported	1,896	5,726	5,393	*
Direct Blue 76	Produced	*	58,000	*	*
	Imported	44,000	250	250	*
Direct Blue 98	Produced	*	139,000	278,000	*
	Imported	*	*	*	*

Table 6. Production and Import Data for Dyes Based on Benzidine and Its Congeners (cont.)

Dye		1975	1976	1977	1978
Based on dichlorobenzidine:		(lb)	(lb)	(lb)	(lb)
Pigment Orange 13	Produced	209,000	267,000	230,000	*
	Imported	11,022	2,200	9,483	3,300
Pigment Orange 34	Produced	99,000	89,000	75,000	*
	Imported	*	96,470	13,420	7,700
Pigment Yellow 12	Produced	6,028,000	7,830,000	8,870,000	*
	Imported	62,117	34,650	28,705	2,256
Pigment Yellow 13	Produced	240,000	380,000	367,000	*
	Imported	2,500	19,984	4,625	9,660
Pigment Yellow 14	Produced	1,840,000	3,000,000	3,248,000	*
	Imported	11,055	*	110	5,192

Source: All data except the 1978 import figures are from Powell et al. (1979). Import figures for 1978 are from USITC (1979).

*Figures are not provided if fewer than three companies produce or import a chemical or if one company is dominant.

well as a significant increase in imports (although total consumption decreased slightly). An increase in U.S. sales of dichlorobenzidine-based pigments (50 percent increase over a 2-year period) can also be discerned. There appear to have been no significant changes in total consumption of dyes and pigments based on o-tolidine and dianisidine in the past few years.

Table 7. Estimated Quantities of Dyes Consumed in U.S.

Type of dye	Quantity (10^6 lb)			
	1975	1976	1977	1978
Benzidine-based:				
U.S. sales	4.2	6.6	4.6	1.9
Imports	0.9	0.6	1.3	1.6
o-Tolidine-based:				
U.S. sales	2.1	2.3	1.9	2.8
Imports	0.1	0.1	0.1	0.1
Dianisidine-based:				
U.S. sales	0.5	0.5	0.4	0.4
Imports	0.1	0.1	0.1	0.1
Dichlorobenzidine-based:				
U.S. sales	8.4	11.6	12.8	---
Imports	0.1	0.1	0.05	0.03

Source: Data on benzidine-, o-tolidine-, and dianisidine-based dyes are from Powell et al. (1979). Data on dichlorobenzidine-based pigments are from USEPA (1979a).

II. HEALTH EFFECTS

A. Benzidine and Benzidine-Based Dyes

1. Mutagenicity

Benzidine was found to be mutagenic in two studies using the Salmonella/mammalian microsome system developed by Ames (Ames et al. 1973, Ferretti et al. 1977). In the Ames study, the maximum mutagenic effect was obtained by the addition of 50 ug benzidine to Salmonella typhimurium TA 1538, in the presence of S-9 mix (a mixture of rat liver homogenate and TPNH-generating system). This treatment produced 265 revertant colonies, compared with 16 and 36 colonies on plates lacking S-9 mix and benzidine, respectively. In a survey of compounds currently used in clinical hemoglobin determinations, Ferretti et al. (1977) confirmed that benzidine is mutagenic when activated by rat liver homogenate.

Benzidine also has been used as a positive control in testing the bacteriocide Grotan BK, a cutting oil preservative (Urwin et al. 1976). The test system was the micronucleus test in rats, in which treated and untreated animals are examined for the presence of micronucleated erythrocytes in bone marrow. Rats administered a total dose of 410 mg/kg benzidine by either dermal or subcutaneous routes developed a content of micronucleated erythrocytes nearly 100-fold higher than controls.

The benzidine-based dyes Direct Red 28 (Congo Red) and Direct Violet 1 (Chlorazol Violet N) were found to be mutagenic when tested by a modification of the Salmonella/microsome system (Sugimura et al. 1977). The dyes were mixed with bacterial cells and microsomes in DMSO and incubated for 20 minutes at 37° C before plating. It was stated that the preincubation was performed because it was a necessary step for obtaining mutagenic activity with potential carcinogens such as dimethylamino-azobenzene and dimethylnitrosamine.

2. Carcinogenicity

The evidence implicating benzidine as a carcinogen in animals and in man has been reviewed by the International Agency for Research on Cancer (IARC 1972a). The number of animal carcinogen studies is extensive; the results of these studies led the IARC to conclude that "benzidine is carcinogenic in the mouse, rat and hamster, and possibly the dog. Given orally, it has produced bladder carcinoma in the dog after a long latent period and liver tumors in the rat and hamster" (IARC 1972a, p. 84).

On the basis of epidemiological studies, the IARC (1972a) concluded that there is a strong correlation between occupational exposure to benzidine and bladder cancer in humans. This conclusion is supported by the study of Zavon et al. (1973) showing that, over a 13-year period, 13 of 25 production workers exposed to benzidine at a dye manufacturing plant subsequently developed bladder cancer. This study was initiated in 1958 when the authors were alerted by the manufacturer that a worker had developed hematuria. At this time, it was not generally known that

benzidine is a human carcinogen, and the plant was still manufacturing benzidine as it had been since 1929, with only a few minor modifications. A detailed investigation was immediately undertaken of the exposure levels of benzidine, the work practices at the plant, and the medical condition of production workers potentially exposed to benzidine. Shortly after this initial investigation, production of benzidine was discontinued. A follow-up study of 25 exposed workers 13 years later disclosed that in the interval 13 had developed bladder tumors. Although multiple exposures to other suspect chemicals did occur, benzidine was the only chemical to which all were exposed, and benzidine thus was judged to be the most likely carcinogen. The time of exposure to benzidine in those who developed tumors ranged from 6-28 years (average 13.6 years), and concentrations of benzidine at various work locations at the time the study was initiated (1958), ranged from 0.005 mg/m^3 to 0.415 mg/m^3 (at six sites). A value of 17.6 mg/m^3 was found at one location, where benzidine was hand shoveled into drums. Benzidine also was found in the urine of 33 workers (including the 25 production workers), before and after work shifts. Average values were about 0.010 mg/1 before work shifts and 0.030 mg/1 after work shifts. The EPA Office of Water Planning and Standards has used these values in estimating that the total accumulated dose of benzidine required to produce a 50 percent incidence of cancer (13/25 observed incidence) is 200 mg/kg (USEPA 1976b).

There is less information available about the carcinogenicity of benzidine-based dyes than there is for benzidine. Following the finding that four benzidine-based dyes (Direct Black 38, Direct Blue 6, Direct Brown 95, and Direct Red 28) are metabolized to free benzidine in rhesus monkeys (Rinde and Troll 1975), the National Cancer Institute (NCI) performed carcinogen bioassays on Direct Black 38, Direct Blue 6, and Direct Brown 95 (NCI 1978a). All three dyes produced tumors in Fischer 344 rats in 13 weeks when included in the diet, but results differed in males and females. Males fed Direct Blue 6 or Direct Black 38 at 1,500 ppm showed a highly significant incidence of hepatocellular carcinomas and neoplastic nodules of the liver. Doses of 1,500 ppm or 3,000 ppm Direct Brown 95 were lethal to males at 5 weeks; lower doses did not produce neoplasms. Females given 3,000 ppm and 1,500 ppm Direct Brown 95 developed hepatocellular tumors and neoplastic nodules of the liver; females given similar concentrations of Direct Black 38 showed no hepatocellular carcinomas but had a significant incidence of neoplastic nodules of the liver. The time-to-tumor interval was 5 weeks for each of the dyes. (For purposes of comparison, it can be noted that rats fed benzidine as 0.017 percent of the diet for 424 days were reported to show an increased incidence of tumors of the liver and bile ducts [Boyland et al. 1954]). In this study, no carcinomas were produced in B6C3F1 mice fed the same dyes at doses ranging from 750 ppm to 12,500 ppm.

3. Metabolism and Bacterial Degradation

The metabolism of benzidine, the benzidine congeners, and their derivative dyes and pigments is of interest because of the reported metabolic conversion of some benzidine-based dyes to the demonstrated carcinogen, benzidine. Haley (1975) presents a comprehensive review of the biotransformation of benzidine and its congeners, in which it was shown that the pattern of metabolites excreted in urine differs among mammalian

species. A summary of the patterns of metabolites excreted in urine by various species following administration of benzidine (route not specified) is shown in Table 8.

The azo linkage in benzidine-based dyes is susceptible to anaerobic enzymatic attack in mammalian species; azo-reductase activity is associated with cytochrome P-450, which occurs predominantly in the liver but also is present in most other organs of the body (Walker 1970). Under anaerobic conditions, this enzyme can catalyze the cleavage of benzidine-based dyes to release free benzidine. Once released, benzidine is converted to other metabolites that may be the active species in carcinogenesis and mutagenesis. The ability of intestinal bacteria to reduce azo-containing dyes also is well established (Walker 1970, Chung et al. 1978).

Table 8. Urinary Metabolites of Benzidine

Compound	Dog	Rat	Mouse	Rabbit	Guinea pig	Man*
Benzidine	+	+	+	±	±	+
4-Acetamido-4-aminodiphenyl	0	+	±	±	±	-
3-Hydroxybenzidine (ether extracts)	+	+		±		+
4,4'-diamino-3-diphenylyl hydrogen sulfate	+	+	±	0	0	-
4'-Acetamido-4-amino-3-diphenylyl hydrogen sulfate	0	+	+	+	+	-
4'-Amino-4-diphenylyl sulfamic acid	0	±	0	+	±	-
N-Glucuronides	+	+	±	+	+	-
Acid-stable unknowns	1	3	3	3	0	-

Source: Adapted from IARC (1972a), Haley (1975). Metabolites were detected and identified by paper chromatography. Although not specified in the references, symbols are interpreted as + = substance detected with certainty; ± = substance presumed to be detected, but not with certainty; and 0 = substance sought but not detected, within limits of technique.

*Also found in man are 3,3'-dihydroxybenzidine, mono- and diacetylbenzidine, and N-hydroxy acetylaminobenzidine.

Four benzidine-based dyes (Direct Black 38, Direct Red 28, Direct Blue 6, and Direct Brown 95) have been subjected to in vivo metabolism studies in rhesus monkeys (Rinde and Troll 1975); the metabolic pathways in this species closely resemble those in humans. Each dye was administered in a

single dose by stomach intubation, and after 72 hours urine was collected and analyzed for free benzidine and monoacetylbenzidine. All four dyes gave similar results, namely, about 1.25 percent of the administered dye was excreted as benzidine (versus 1.45 percent benzidine excreted when a comparable dose of benzidine was administered). The amount of free benzidine recovered was much greater than was present as impurities in the dyes administered. NCI performed similar metabolism tests on rodents with three of these dyes--Direct Black 38, Direct Brown 95, and Direct Blue 6--and also found free benzidine and monoacetylbenzidine excreted in the urine (NCI 1978a).*

Feeding studies with additional dyes are under way in dogs at the National Institute of Environmental Health Sciences (NIEHS); dyes are administered orally at a dose of 100 mg/kg body weight and urine is collected for 72 hours and examined for free benzidine using chromatography-mass spectrometry (Matthews 1979). In preliminary results obtained for six benzidine-based dyes (Direct Blue 2, Direct Black 4, Direct Brown 2, Direct Red 28, Direct Orange 8, and Direct Green 1) benzidine was excreted in urine in amounts that exceeded by at least 25-fold the free benzidine present as contaminants in the administered dyes. It appears, therefore, that conversion of benzidine-based dyes to benzidine may be a generalized phenomenon in the dog. If a similar conversion occurs in humans, these dyes may have the potential to induce human cancer.

With regard to the latter statement, a recently completed study conducted by the National Institute for Occupational Safety and Health (NIOSH) suggests that benzidine-based dyes can be metabolized to free benzidine in humans (Boeniger 1979). Workers at a textile plant using benzidine-based dyes were found to excrete benzidine in their urine at concentrations as high as 0.039 mg/l (39 ppb), much higher than could be attributed to the free benzidine present as a contaminant in the dyes. The finding that benzidine-based dyes can be metabolized to a known carcinogen in humans is cause for concern; the fact that the concentration of benzidine detected was higher than the average values of 0.010-0.030 mg/l (10-30 ppb) found in 13 of 25 dye production workers who subsequently developed bladder cancer (Zavon et al. 1973) is especially alarming.

Although multiple exposure routes may be postulated for workers exposed to benzidine-based dyes (i.e., inhalation, dermal, ingestion through hand contamination), a recent report from industry suggests that dermal absorption may be a specific problem. A preliminary report, filed

*Highly polar compounds, however, are not well absorbed from the gut and thus water-soluble sulphonated dyes such as the majority of commercially available benzidine-based dyes would not be expected to be well absorbed by mammals (Walker, 1970). Therefore, one can postulate that reductive cleavage of benzidine-based dyes would be expected to occur mainly by the gut flora. Oral administration of benzidine-based dyes in animals with subsequent testing of urine for free benzidine would not distinguish among the two mechanisms for reductive cleavage of the azo linkages.

under section 8(e) of the Toxic Substances Control Act* suggests that the benzidine-based dye Direct Black 38 is absorbed through the skin in rabbits (International Business Machines, 1979). Rabbits were dosed dermally with dye (radioactively labeled with ^{14}C in the biphenyl moiety) in a proprietary solution. Over a period of 144 hours, 91 percent of the applied radioactivity was recovered in the urine. Because a detailed test protocol was not submitted, this report can only be considered suggestive at present; however, it is noteworthy as the first report of the absorption of a benzidine-based dye through the intact skin of a mammal.

B. Dichlorobenzidine and Dichlorobenzidine-Based dyes

1. Mutagenicity

Dichlorobenzidine (50 µg/plate) was weakly mutagenic in the Ames Salmonella assay without S-9 mix (114 revertant colonies versus 8 colonies on control plate, Garner et al. 1975). In the presence of S-9 mix, the mutagenic activity was about thirtyfold higher (3,360 revertant colonies).

2. Carcinogenicity

Dichlorobenzidine has been reviewed by the International Agency for Research on Cancer (IARC 1973a) and found to be a carcinogen in the rat and hamster. Studies that support the IARC's conclusion are described below.

In a 12-month study of rats fed 10-20 mg dichlorobenzidine in the diet six times per week (total dose 4.5 g/rat), a high incidence of tumors of the Zymbal gland and other organs was found (Pliss 1959). Subcutaneous administration of 15-60 mg dichlorobenzidine in sunflower seed oil or glycerol and water (at unspecified intervals) to rats for 10-13 months gave rise to tumors in about 75 percent of the animals. Tumors of the skin, mammary glands, and sebaceous glands were most numerous; intestinal, urinary bladder, and bone tumors also were observed. One of 25 control rats, injected with the vehicle alone, developed a sarcoma.

Stula et al. (1971, 1975) fed 50 male and 50 female ChRCD rats 1,000 ppm dichlorobenzidine in a standard diet for up to 16 months and observed malignant tumors of the mammary glands, skin, and acoustic ducts in both sexes. Treated males also had an increased incidence of haemopoietic tumors, compared to an equal number of controls.

Dietary levels of 0.1 percent dichlorobenzidine in lifetime feeding studies, however, did not induce tumors in 30 male and 30 female Syrian golden hamsters, when compared with a similar number of controls. Dietary levels of 0.3 percent dichlorobenzidine, however, produced four transitional cell carcinomas of the bladder and some liver cell tumors among 60 test animals; no such tumors were observed in an equal number of control animals (Saffiotti et al. 1967, Sellakumar et al. 1969).

*Under section 8(e) of TSCA information that a chemical may present substantial risk must be submitted to the Administrator of the U.S. Environmental Protection Agency.

Finally, a study by Golub and Kolesnichenko (1974) is of theoretical interest. Dichlorobenzidine was administered to mice during the last week of pregnancy; the progeny were observed for 10-20 months. A significant increase in tumors occurred in the progeny, suggesting that dichlorobenzidine may be a transplacental carcinogen.

At the time of the review of dichlorobenzidine by the IARC, epidemiological studies in humans had not been performed. Because benzidine and dichlorobenzidine were commonly produced at the same plant, the possibility could not be excluded that dichlorobenzidine had contributed to the incidence of bladder cancer commonly attributed to benzidine. In 1974, Gerarde and Gerarde presented an epidemiological study of 175 out of 207 persons exposed to dichlorobenzidine over a period of 35 years; no cases of bladder tumors were found. However, this study was published without review, owing to the untimely deaths of the authors, and a number of questions concerning the reliability of the data and its significance (considering that no medical information was available for 32 workers) necessarily remain unanswered (see Commentary section appended to Gerarde and Gerarde 1974). Another serious limitation of the work is the fact that data on bladder tumors only were reported, although dichlorobenzidine is known to produce tumors of other organs in animals (Pliss 1959, 1963; Sellakumar et al. 1969).

MacIntyre (1975) also reported the lack of incidence of bladder tumors among 225 British dye workers exposed to dichlorobenzidine for up to 30 years. Since 1965 it has been the practice at this plant to give a medical examination to exposed workers regularly and to do Papanicolau smears every 6 months. In this particular study, most workers were first exposed fewer than 20 years ago, and most have been exposed for fewer than 16 years (the latency period for benzidine-induced urinary bladder cancer is about 16 years). Furthermore, work practices at the plant since the late 1950s have greatly minimized the extent of actual exposure. All of these factors tend to diminish the force of the negative findings with respect to bladder tumors.

In addition to the above epidemiological study, MacIntyre (1975), citing unpublished information presented at a scientific meeting in 1974, noted that occupational physicians in Europe had found no bladder cancer among some 1,000 persons exposed to dichlorobenzidine. Thus, the only conclusion that can be drawn from the accumulated weight of negative findings is that if dichlorobenzidine is a bladder carcinogen in humans, it is probably much less potent than benzidine.

Animal studies also have been performed on some dichlorobenzidine-based pigments. In a study at NCI, a technical grade of Pigment Yellow 12 was fed to Fischer 344 rats and B6C3F1 mice for 78 weeks, followed by observation for 18-22 weeks (NCI 1978b). High-dose animals received the pigment as 5 percent of their diet; low-dose animals received it as 2.5 percent of the diet. Treated rats showed no increase in the incidence of neoplasms; however, a statistically insignificant increase in the following neoplasms were observed: metastatic chordoma in 1/49 low-dose males and an osteogenic sarcoma in 1/49 low-dose females. Treated mice also showed no statistically significant increase in the incidence of neoplasms, although three specific findings were noted: squamous cell carcinomas of the ear in

1/49 low-dose males, an infiltrating duct carcinoma of the mammary gland in 1/50 low-dose females, and a mastocytoma of the subcutaneous tissue in 1/50 high-dose females. These tumors were not found among the controls, although a comparable incidence of other tumors was found. The study concluded that the results did not provide evidence for the carcinogenicity of Pigment Yellow 12 in Fischer 344 rats or B6C3F1 mice.

In another study, mice (NMRI, Ivanovas) and rats (Sprague-Dawley, Ivanovas) were fed Pigment Yellow 12 (containing 2 ppm dichlorobenzidine) in the diet at concentrations of 0.1, 0.3, and 0.9 percent for 104 weeks (Leuschner 1978). Test groups and controls consisted of 50 males and 50 females. No significant incidence of tumors was found in treated animals, compared with controls. Similar results were obtained in this study (following a similar protocol) with Pigment Yellow 83, another dichlorobenzidine-based pigment.

3. Metabolism

Sciarini and Meigs (1961) followed the biotransformation of dichlorobenzidine in a mongrel dog injected intraperitoneally with 1 gm dichlorobenzidine suspended in gum tragacanth. The only recovery product identified in urine and feces was dichlorobenzidine; about 2 percent of the administered dose was excreted over a 15-day period, primarily (90 percent) in feces. As the chemical tests applied should have detected metabolites similar to those produced by benzidine, it was concluded that dichlorobenzidine is metabolized by a different route, or not at all, in the dog.

Kellner et al. (1973) compared the distribution and elimination of dichlorobenzidine and benzidine in rats, dogs, and monkeys after intravenous injection. In all cases, dichlorobenzidine was excreted much more slowly than was benzidine. In monkeys, unchanged dichlorobenzidine was found in the urine a few hours after injection, whereas nearly all the injected benzidine found in the urine was present as metabolites rather than unchanged benzidine. These results support the view of Sciarini and Meigs (1961) that dichlorobenzidine is metabolized quite differently from benzidine.

A number of studies on the metabolic fate of dichlorobenzidine-based pigments fail to provide any evidence that these pigments are broken down to release free dichlorobenzidine. These included an NCI study (unpublished) in which rats were fed Pigment Yellow 12 by stomach intubation and the study by Leuschner (1978) in which rabbits were fed a single dose of Pigment Yellow 12. (The protocol of Leuschner's study duplicated that of an earlier study by Akiyama [1970], which reported that metabolic release of dichlorobenzidine from Pigment Yellow 13 did occur.) The preceding studies are supported by the work of Stavenuiter (1977), who detected practically no free dichlorobenzidine in urine following injection of ^{14}C-labeled Pigment Yellow 13 to rats and rabbits. Thus, the weight of the evidence supports the view that dichlorobenzidine-based pigments are only poorly (if at all) metabolized to dichlorobenzidine in mammals. Leuschner (1978) attributed the poor metabolism of dichlorobenzidine-based pigments (Yellows 12, 13, and 83) and one o-tolidine-based pigment (Yellow 16) to poor absorption of the pigment particles (0.1=1.0 μm in size). Other

explanations, however, may be based on the structural differences between benzidine-based dyes and these pigments. Dichlorobenzidine obviously differs from benzidine in that chlorines are substituted for hydrogens in the 3 and 3'-positions of the molecule. A more significant structural difference, however, may be the substituent group in the pigments adjacent to the azo linkage. For comparison, Figure 6 shows the structures of the eight benzidine-based dyes known to be metabolized to benzidine and the three dichlorobenzidine-based pigments found not to be metabolized to dichlorobenzidine. Also shown is the o-tolidine-based pigment, Pigment Yellow 16, which was found not to be metabolized to o-tolidine in mice and rats (Leuschner 1978). As can be seen, all of the pigments, but none of the dyes, contain the substituent $R-C=COH-CH_3$ adjacent to the azo linkage. This group is potentially capable of keto-enol tautomerism and of forming a resonant ring structure, as shown in Figure 7. If the keto form predominates or exists exclusively, it is probable that these pigments, lacking an azo linkage, would be impervious to enzymatic reduction. A similar explanation was advanced by Jones et al. (1963) to account for the stability of several 4-arylazo-5-pyrazolones to enzymatic azo-reduction. In the latter study, infrared and N.M.R. spectroscopy revealed that the pyrazolones exist exclusively in a keto form that is stabilized by intramolecular hydrogen bonding and therefore lack an azo linkage (Figure 8). If correct, this hypothesis (with respect to Pigments Yellow 12, 13, and 83) may also account for the lack of carcinogenicity of the dichlorobenzidine-based pigments.*

C. o-Tolidine

1. Mutagenicity

o-Tolidine was found to be weakly mutagenic in the Ames Salmonella assay system with S-9 activation (Feretti et al. 1977). Under these conditions, addition of 100 µg o-tolidine produced 80 revertants/plate, compared with 6 revertants/plate for the control lacking S-9 mix.

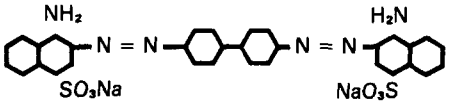
Trypan Blue, an o-tolidine-based dye, was found to be mutagenic to Aspergillus (Cooke et al. 1970, Roper 1971) and Salmonella (Hartman et al. 1978). The commercial grade of Trypan Blue contains a significant amount of monoazo dyes (containing substituted diphenyl groups other than o-tolidine) as impurities (Field et al. 1977); the samples of dye used in the mutagenicity studies reported here were of unspecified purity. In order to increase the sensitivity of the Ames Salmonella system to Trypan Blue, Hartman et al. (1978) determined the experimental conditions necessary for obtaining azo dye reduction in cell-free extracts of the intestinal anaerobe, Fusobacterium sp. 1. Maximum azoreductase activity required FMN, glucose-6-P, and anaerobic conditions. When 500 µg Trypan Blue was subjected to the azoreductase regimen, then tested in the Ames

*The possibility that dichlorobenzidine or pigments based on it can be converted to benzidine in vivo is considered remote. The loss of halogens from the benzene ring in vivo has never been reliably demonstrated (Gerarde and Gerarde 1974).

DYES

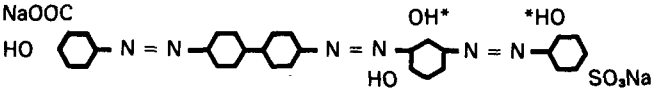
C.I. Direct Red 28 *(Yellowish red)*

Classical name Congo Red

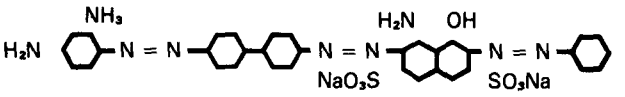


C.I. Direct Brown 95 *(Reddish brown)*

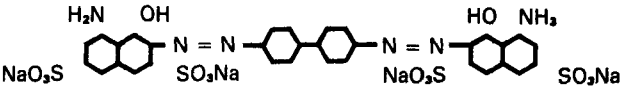
Copper complex derived from



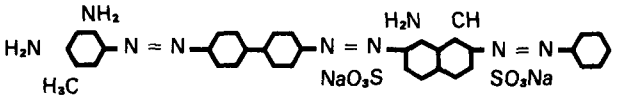
C.I. Direct Black 38 *(Black)*



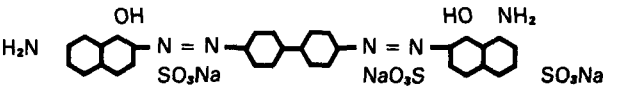
C.I. Direct Blue 6 *(Blue)*



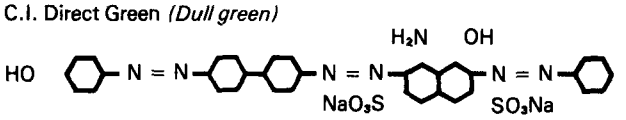
C.I. Direct Black 4 *(Black)*



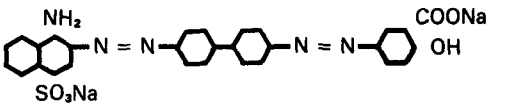
C.I. Direct Blue 2 *(Dull blue)*



C.I. Direct Green *(Dull green)*

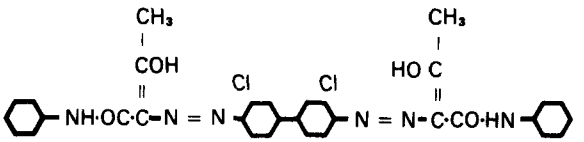


C.I. Direct Orange 8 *(Reddish orange)*

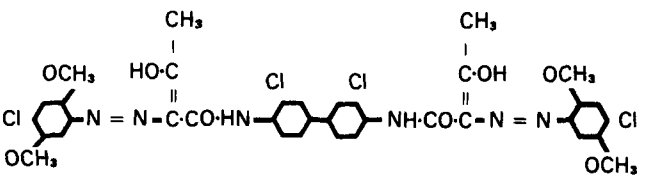


PIGMENTS

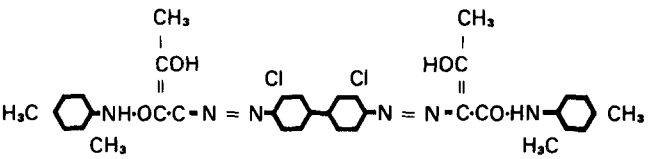
C.I. Pigment Yellow 12 *(Yellow)*



C.I. Pigment Yellow 83



C.I. Pigment Yellow 13 *(Yellow)*



C.I. Pigment Yellow 16 *(Greenish yellow)*

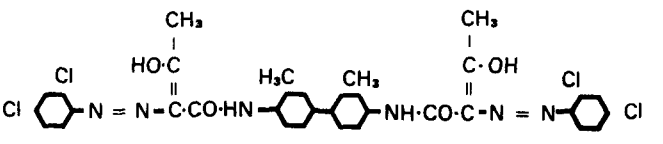


Figure 6. Comparison of structures of dyes and pigments.

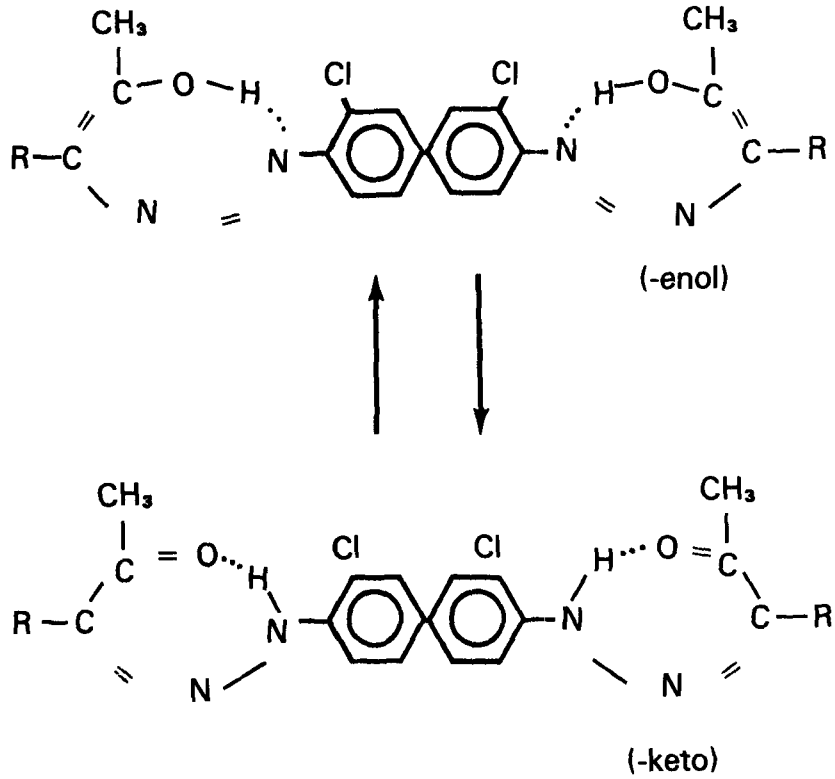


Figure 7. Proposed keto-enol tautomerism of dichlorobenzidine-based pigments, with presumed ring structures.

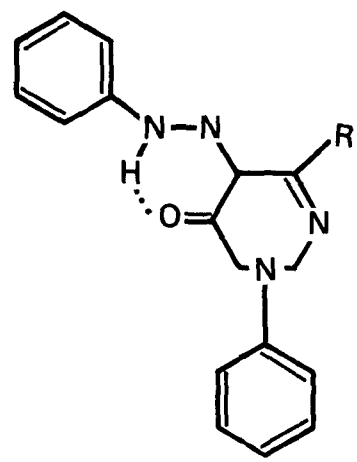


Figure 8. Structure of hydrazone form of arylazopyrazolones, as determined by Jones et al. (1963).

Salmonella system with S-9 activation, it was found to be clearly mutagenic (118 revertant colonies on treated plates versus 39 revertant colonies on control plates). A similar amount of Trypan Blue, not subjected to the azoreductase pretreatment, was not mutagenic, with or without S-9 activation.

2. Carcinogenicity and Teratogenicity

o-Tolidine was reviewed by the IARC (1972b) and was judged to be a carcinogen in rats when injected subcutaneously. In one study, Spitz et al. (1950) injected 105 Sherman rats with commercial o-tolidine in olive oil (at weekly intervals) in doses of 60 mg/rat per week (total dose 5.5 g). Only 48 rats survived more than 300 days; these were kept for the remainder of their life span. Five of the 48 surviving rats developed cancer of the auditory canal. Although no concurrent controls were run, none of the 578 untreated rats of the same colony were found to have similar cancers (the control group had a total of 56 other tumors).

In a second study, random-bred rats were given weekly subcutaneous injections of purified o-tolidine in sunflower seed oil (20 mg per rat per week) for 13 months. Tumors first appeared at 8 months; among 50 treated animals 30 developed a total of 41 tumors, including 2 carcinomas of Zymbal's gland (Pliss and Zabezhinsky 1970). Only one of 50 control rats injected subcutaneously with sunflower seed oil alone developed a tumor, a sarcoma associated with a parasitic cyst. Another group of 88 rats (equal numbers of males and females) was given weekly subcutaneous implants of 20 mg purified o-tolidine in 10 mg glycerol for 13 weeks. In about half the animals (equal numbers of males and females) the o-tolidine was subjected to ultraviolet irradiation prior to injection; no significant effect of this treatment was observed. First tumors appeared at 11-12 months, and 48 of the 68 surviving animals developed a total of 60 tumors, including 27 Zymbal's gland carcinomas. No concurrent controls were run in this study, but it was reported that the strain of rats used had a low incidence of spontaneous tumors (Pliss 1965).

No human epidemiological studies are available for o-tolidine (IARC 1972b). Several case studies of cancer in workers manufacturing o-tolidine and benzidine are summarized in a report published by NIOSH (1978a). No cases of cancer in workers exposed solely to o-tolidine have been reported, possibly because both chemicals historically have been handled at the same factory sites.

One carcinogenicity study was found in the available literature on an o-tolidine-based pigment, Pigment Yellow 16 (Leuschner 1978). Rats fed the pigment (containing less than 1 ppm o-tolidine) as part of the diet (in concentrations of up to 0.9 percent) for 104 weeks had no higher incidence of tumors than did controls.

Trypan Blue, an o-tolidine-based dye, also has been tested for carcinogenicity in rats (Field et al. 1977). Inbred Wistar rats were injected at biweekly intervals with dye in water solution (5 or 10 mg per injection) for up to 40 weeks. The commercial grade dye was tumorigenic (5 tumors among 17 rats); however, the crude dye contained a significant proportion of monoazo dyes containing substituted diphenyl groups other

than o-tolidine. When the o-tolidine component was purified and tested no tumors were observed, but precancerous changes were reported in 11 of 33 rats (Field et al. 1977). From these results it is not possible to determine the oncogenic potential of the pure o-tolidine-based dye component.

Both crude and purified Trypan Blue were found to be potential teratogens in mice in the preceding carcinogen study. Specific pathogen-free CFLP mice were injected with dye at about 7.5 days of pregnancy and were killed and examined on day 18 of pregnancy. Mice dosed with crude or pure Trypan Blue (60 or 120 mg/kg body weight) gave a significantly higher incidence of resorptions and major malformations than did controls injected with saline.

3. Metabolism

The metabolism of o-tolidine has been reviewed in a report by NIOSH (1978b). The available evidence indicates that in humans o-tolidine is metabolized by a pathway similar to that of benzidine, with the first steps being the acetylation of the amino groups and introduction of the phenolic group on the aromatic ring. Urine of workers occupationally exposed to o-tolidine has been reported to contain o-tolidine, N,N'-diacetyl-o-tolidine, and 5-hydroxyl-o-tolidine and its conjugates (NIOSH 1978b).

Leuschner (1978) examined the urine of rats fed the o-tolidine-based pigment, Pigment Yellow 16, for evidence of reductive metabolism; no o-tolidine was found (detectable limit was 0.3 ppm). Currently, o-tolidine-based dyes are being tested for reductive metabolism in dogs (Matthews 1979). Although no evidence is currently available from these mammalian metabolism studies, the reader should note that intestinal bacteria can release o-tolidine from o-tolidine-based dyes (Hartman et al. 1978).

D. Dianisidine

1. Mutagenicity

Dianisidine has been found to be weakly mutagenic in the Ames Salmonella assay, but only with microsomal activation (Garner et al. 1975).

2. Carcinogenicity

Dianisidine has been reviewed by the IARC (1973b) and was judged to be a carcinogen in rats when administered orally. In one experiment, rats were administered 30 mg dianisidine in sunflower seed oil 3 times per week for 13 months (Pliss 1965). Of the 18 rats surviving this treatment, 4 developed tumors not found in the controls. The tumors included two Zymbal gland tumors, one ovarian tumor, and one fibroadenoma of the mammary gland. None of the 50 control rats developed tumors at these sites.

In another study, Fischer rats were administered various doses (up to 30 mg/animal) of dianisidine by stomach tube 5 days a week for 52 weeks (Hadidian et al. 1968). A significant number of the 30 male and 30 female rats treated in this manner developed tumors that were not found among the

360 control animals given the vehicle alone. Tumors occurred at various sites including the bladder (two papillomas), the intestine (three carcinomas), the skin (five carcinomas) and the Zymbal gland (three carcinomas). No differences between sexes were noted.

No epidemiological data on the occurrence of cancer in workers exposed to dianisidine in the absence of other suspect carcinogens were found in the available literature.

III. ECOLOGICAL EFFECTS

Little information was found concerning the possible ecological effects of benzidine. No reports on the effects of benzidine or its congeners in the natural environment were found. In a letter to Dr. Donald I. Mount, Director, National Water Quality Laboratory (EPA), Duluth, Minnesota, November 20, 1973, A. E. Lemke described laboratory studies in which it was determined that benzidine, at concentrations of a few ppm, is acutely toxic to six species of fish (see Table 9). A study by the Synthetic Organic Chemical Manufacturers Association (SOCMA) reported the acute toxicity of benzidine to three additional fish species (SOCMA 1975). This study is reviewed in the Criteria Document on benzidine, issued by the U.S. EPA Office of Water Planning and Standards (USEPA 1976). The TL_{50} values reported in this document are listed in Table 9.

In an uptake and elimination study using C^{14}-labeled benzidine, bluegill sunfish suffered 8 percent mortality from exposure to 98 ppb benzidine for 42 days (EG and G Bionomics 1975). Benzidine, at this concentration, was found bioaccumulated 44-fold in muscle tissue at 21 days; this level of bioaccumulation remained constant throughout the remainder of the 42 days of exposure.

A recent study indicates that dichlorobenzidine bioconcentrates into both the edible and nonedible portions of bluegill sunfish (Sikka et al. 1978). Mortality occurred before equilibrium between water and fish was attained; the bioconcentration factors reached at mortality were 135-fold to 554-fold. These values, as well as the 44-fold bioaccumulation found with benzidine, are well below the 5,000-fold bioaccumulation factor currently considered by EPA to constitute substantial risk to the environment.

No information was found on the potential ecological effects of other benzidine congeners (dianisidine and o-tolidine) or any of the dyes or pigments derived from benzidine and its congeners.

Table 9. TL_{50} Values, Benzidine (ppm)

	(Static bioassay)			
Species	24 hr	48 hr	72 hr	96 hr
Flagfish*	>50	32.5	25.0	16.2
(Jordanella floridae)				
Fathead Minnow*	>20	>20	>20	>20
(Pimephales promelas)				
Fathead Minnow†	-	-	-	-
Red Shiner*	>20	10	2.5	2.5
(Notropis lutrensis)				
Lake Trout*	8.7	5.	4.35	4.35
(Salvelinus namaycush)				
Rainbow Trout*	>20	14.1	10	7.4
(Salmo gairdneri)				
Scud*	>20	>20	>20	>20
(Gammarus pseudolimaeus)				
Emerald Shiners†	-	-	-	5
(Notropis atherinoides)				
Bluegill Sunfish†	-	-	-	15
(Lepomis macrochirus)				

*A. E. Lemke in a letter to Dr. Donald I. Mount, Director, National Water Quality Laboratory (EPA), Duluth, Minnesota, November 20, 1973.

†USEPA (1976)

IV. ENVIRONMENTAL FATE

A. Water

The most important source of environmental release of benzidine and benzidine congeners is probably the wastewaters from dye producer plants. In the absence of any reported studies of persistence in water, Radding et al. (1975) estimated the half-life of benzidine in water to be about 100 days. This estimate assumed that degradation would proceed mainly through reaction with peroxy radicals formed by the action of sunlight on water. Because the rate constants used for this calculation were only estimates, the actual half-life of benzidine may be much greater or smaller. Dichlorobenzidine is very rapidly degraded in aqueous solutions by the action of natural or simulated sunlight (Sikka et al. 1978). Benzidine and 3-chlorobenzidine are intermediates in this process. Although the mechanism of dechlorination is unknown, it is known not to involve carbon-chlorine bond homolysis. In organic solvents the dechlorination reaction is considerably slower (Sikka et al. 1978).

Benzidine is resistant to biooxidation by unacclimated microorganisms in activated sludge (Taback and Barth 1978). Keinath (1976) reported that, in an unacclimated activated sludge system, benzidine is biodegradable at 0.05 mg/l but not at 1 mg/l. To determine whether microorganisms in sludge can become acclimated to benzidine and can adapt enzymes to metabolize it aerobically, Taback and Barth (1978) subjected sludge microorganisms to benzidine in aerobic growth chambers for up to 9 weeks. A reservoir of wastewater containing sludge was seeded with benzidine and refrigerated, then fed through the aerobic growth chamber at a rate set to attain a hydraulic retention time of 24 hours in the chamber. The results indicate that acclimation to up to 5 mg/l benzidine was readily attained; the concentration of residual benzidine in effluent from the reactor was reduced to 2.2 mg/l after 1 week and to 0.3 mg/l after 7 weeks. At 1 mg/l input concentration, residual benzidine dropped to 0.09 mg/l after 1 week, and thereafter was undetectable (0.001 mg/l, or 0.1 percent of starting concentration, could have been detected by the test method used).

A 1976 study of wastewater treatment methods disclosed that housekeeping measures available to benzidine manufacturers (e.g., oxidation with ozone or nitrous acid) are adequate to prevent the discharge of benzidine directly into sewer lines (Keinath 1976). However, the effects of possible by-products of these processes have not been assessed. Benzidine concentrations can be reduced by 1-10 ppb by adsorption on granulated charcoal. Analysis for benzidine in the exhausted dye liquids from a textile industry making heavy use of benzidine-based dyes gave an average concentration of 3.5 ppb. Similar mass balance analyses conducted for "heavy benzidine-dye use" leather and manufacturing concerns showed calculated residual benzidine concentrations of 0.25 and 3.5 ppb, respectively (Keinath 1976). All of these values are well below the 50 ppb limit for biodegradation by unacclimated activated sludge (Keinath 1976).

Dichlorobenzidine adsorbs extensively to a variety of aquatic sediments and becomes more tightly bound with time (Sikka et al. 1978). Both benzidine and dichlorobenzidine are more soluble in water than is DDT (a well-known bioaccumulator), and all three compounds are also quite soluble in organic solvents. Thus, it is suspected that bioaccumulation occurs when either of these benzidine compounds is present in large bodies of water and that the compounds can move within the food chain. Recent studies indicate that both benzidine and dichlorobenzidine are rapidly bioconcentrated in bluegill sunfish (see section III, Ecological Effects).

Benzidine-based or congener-based dyes and pigments discharged into waters from manufacturing facilities are believed to be chemically reduced back to the parent compound if either hydrogen sulfide or sulfur dioxide is present in the receiving waters. This was believed to be the case downstream of a dye plant on the Sumida River in Japan, where benzidine concentrations of up to 233 ppb were found (Takemura et al. 1965). Hitz et al. (1978) carried out laboratory studies on the adsorption of dyes to activated sludge. Dyes (in concentrations found in effluents received at treatment plants) were mixed with activated sludge at a concentration that is comparable to the concentration in the aeration stage of sewage plants in parts of Britain and the rest of Europe. After 30 minutes the sludge was removed by centrifugation, and the dye remaining in solution or suspension was determined. Average adsorption of seven basic dyes ranged from 50-92 percent; the two dianisidine-based dyes used, Direct Blues 1 and 15, showed 92 and 90 percent adsorption, respectively (no other dyes derived from benzidine or its congeners were tested).

B. Air and Soil

No data were found on the distribution of benzidine in air. Radding et al. (1975) considered the probable principal chemical reactions of benzidine in air to be photolysis and oxidation by ozone; however, lacking any reaction data, they could only estimate crudely the half-life of benzidine in air as approximately one day. In soil, benzidine is probably immobilized rapidly by adsorption to humic material (Radding et al. 1975) and clays (Furukawa and Brindley 1973). The Fe^{+++}, Al^{+++}, and Cu^{++} ions, which are readily available in some clays soils, are believed to oxidize benzidine very rapidly (Furukawa and Brindley 1973).

V. EXPOSURE ANALYSIS

A. Distribution in the Environment

Benzidine and its congeners are not known to occur naturally in the environment, and there is little published information bearing on this point. A review of the literature available in 1978 (Shriner et al. 1978) identified only one study on the distribution of benzidine or its congeners in the natural environment, and in this case only water was examined. In 1973-1975 a field survey was conducted by the Synthetic Organic Chemical Manufacturers (SOCMA) Task Force on Benzidine to determine whether benzidine, suspected of being released by upriver plants, could be detected in the Buffalo, New York, and Niagara River areas (reviewed in Howard and Saxena 1976). Samples of sediment and water were gathered from seven sites upstream and seven sites downstream from a plant producing benzidine-based dyes. No benzidine or benzidine-based salts were detected in any of the samples (detectable limit was 0.2 ppm).

B. Sources of Release to the Environment

1. Benzidine and Its Congeners

Although few actual measurements of release of benzidine compounds into the environment have been reported, it is thought that manufacturing and processing plants for dyes and pigments derived form benzidine and its congeners are the major sources of their release (Shriner et al. 1978). It would be useful to know

(a) the total amount of each dye produced by each manufacturer,
(b) the total number of sites of production for each dye, and
(c) the amount of benzidine or congener released at each site;

however, this information could not be gleaned from available sources.

In the dye-manufacturing process, the major potential routes for release of dye-base compounds are air-borne dust produced during weighing, loading, and mixing operations and wastewaters. Boeniger (1979) made cotton swipes at the following six locations inside a plant that produced benzidine dyes: inside a benzidine azo reactor; at the opening of an azo charging chute; in the bottom area of a diazo tank wall; inside the bottom outlet spigot of a diazo tank; on the doorknob of a changing room used for decontaminating workers; and at the charging chute for hydrazobenzene dumped into a reactor vessel. No benzidine was detected at any of the sites. Because dust would be expected to be present on most of these surfaces, it is unlikely that airborne release is a significant source of benzidine at dye manufacturing sites using procedures similar to the plant in Boeniger's study. No information is available for estimating the extent of release of benzidine congeners by this route.

EPA standards for benzidine and its salts in wastewaters from dye manufacturers were established in January 1977 in the following rule:

"(3)Discharges from a manufacturer shall not contain benzidine concentrations exceeding an average per working day of 10 µg/l calculated over any calendar month, and shall not exceed a monthly average daily loading of 0.130 kg/kkg of benzidine produced, and shall not exceed 50 µg/l in a sample(s) representing any working day" (USEPA 1977). Standards set for dye applicators are similar except that maximum daily concentrations of benzidine in effluents were limited to 25 µg/l, one half the maximum allowed benzidine-dye manufacturers. All standards apply only to wastes discharged directly into streams or rivers; EPA is developing standards for discharge into secondary treatment plants. The only current benzidine-based dye producer (Fabricolor) discharges its wastewaters into municipal wastewater systems; it is believed that the final wastewaters, after biological treatment, probably show no detectable benzidine (Archer et al. 1977).

2. Dyes and Pigments

The Dyes Ecological and Toxicology Organization (DETO) identified the following three major sources for environmental release of dyes and pigments derived from benzidine and its congeners.

(a) Process wastewaters. Although this seems a likely source of release of dyes and pigments, no information was obtained regarding the amounts of dyes and pigments derived from the benzidine and its congeners released into wastewaters, either during the manufacturing process or in dyeing operations. It is known that most manufacturers and dye applicators subject their wastes to secondary treatment plants (memo from DETO to Dr. Fred Clayton, TSCA/ITC vice-chairperson, June 27, 1979) but the effectiveness of these treatments is not known.

(b) Atmospheric release. Release of dyes in the atmosphere as particulate matter can occur during dye manufacturing processes such as drying and grinding, as well as during handling of dyes in application industries (e.g., in drug rooms of textile mills where dyes are weighed out for dye liquor bath preparation). Except in situations in which dusting is considered a serious problem requiring special means of collection, dust particles (including dyes) are collected by ventilators and exhausted into the air outside the plant.

(c) Disposal and dyed articles. Clothing, colored paper, leather, and other dyed goods are commonly disposed into landfill sites as solid wastes. Dyes could be released from these objects into the environment, but--due to the high affinity of the dyes for the substrate on which they are applied--it is thought that any such release from manufactured articles would be a slow process.

C. Population Exposed

1. Industrial Workers

The people most exposed are the workers who manufacture, handle, or use the dyes and pigments derived from benzidine and its congeners. Data

concerning the numbers of such workers and the extent of their exposure ar. generally unavailable but are being compiled by DETO.

Occupational standards instituted by OSHA in 1974 identified benzidine (including its salts) as a carcinogen and, therefore, subject to regulation as a health hazard in the workplace. The standards require that any area in which benzidine is manufactured, processed, used, repackaged, released, handled, or stored be designated a regulated area, with entrance and exit restricted and controlled. Detailed work rules aimed at minimizing exposure are prescribed (e.g., washing and change of clothes required on entering and leaving a regulated area; use of a half-face, filter-type respirator required in regulated areas; use of a hood or an isolated system such as a glovebox when handling benzidine). The standards, however, do not apply to any compounds that contain less than 0.1 percent free benzidine (1,000 ppm) by weight or volume. No safe levels of benzidine in the workplace were established, and environmental monitoring was not required. Presumably these regulations have reduced worker exposure to benzidine, but the actual extent of exposure cannot be assessed at present.

Occupational exposure to benzidine-based dyes is presently under the scrutiny of NIOSH. Based on a national survey from 1972-1974, NIOSH estimated that no fewer than 6,500 workers are potentially exposed to benzidine-based dyes (NIOSH 1979). Boeniger (1979) investigated worker exposure to azo dyes, with special attention directed to those derived from benzidine. This comprehensive study monitored a variety of workers and job sites in two dye-manufacturing plants, two textile plants, a tanning and leather finishing plant, and a paper producing plant. Dye concentrations in the air were determined from particulate matter trapped in filters (both stationary filters and filters attached to workers were used), with subsequent spectroscopic analysis of soluble matter removed from filters. Urine samples over varying lengths of time were collected from workers and analyzed for the presence of total aromatic amines, benzidine, and monoacetylbenzidine (MAB); the presence of these compounds is indicative of metabolism of the dyes. In all, 38 workers potentially exposed to benzidine (representing all 6 sites) were monitored. Highly significant was the finding of benzidine or MAB in quantities ranging from about 1 ppb up to 112 ppb benzidine and 590 ppb MAB in 8 of the 38 workers monitored; 6 of the 8 worked at one of the dye manufacturing plants, and 2 worked at a textile finishing plant. The concentrations of benzidine in urine were found to be higher than could be accounted for by traces of free benzidine measured in the dyes used; thus, by inference, the benzidine and MAB resulted from metabolic conversion of the dyes. The major conclusion of this study is that the number of workers previously thought to have potential exposure to benzidine may be greatly underestimated (Boeniger 1979).

A recent report states that the growing use of imported benzidine dyes may result in increased exposure of dye workers to residual free benzidine, reported to be as high as 500 ppm in some imported dyes, compared to 20 ppm or less in domestically produced dyes, although no actual data were presented (Castleman 1979). To investigate this question, Boeniger (1979) examined 26 randomly chosen imported dyes and an equal number of domestic dyes for residual benzidine content. Results are shown in Tables 10 and 11. Although the highest residual benzidine found was in a domestic dye,

this was the only domestic sample containing more than 20 ppm benzidine, whereas six of the imported dyes exceeded this value. Thus, it seems likely that increased use of imported dyes will lead to an increase in exposure to residual benzidine. Although the amount of increased exposure cannot be determined from the information at hand, it is probably much less than that implied by the value of 500 ppm residual benzidine cited by Castleman (1979).

Table 10. Residual Benzidine in Direct Dyes from Domestic Sources

Company and dyes	Residual benzidine in ppm (w/w)
GAF Corporation	
Black JXA (Blk. 38)	13
Black ER-200 (Blk. 38)	4
Black EA (Blk. 38)	2
Brown BRL (Brn. 95)	2
Blue 2B (Blue 6)	4
Scarlet 4BGP (Red 39)	<1
Fabricolor	
Brown 3GN (Brn. 95)	270
Black GX (Blk. 38)	20
Brown BRL 200% (Brn. 95)	19
Brown 3GN (Brn. 154)	15
Green WS 133% (Grn. 1)	12
Fast Blue 2B 250% (Blue 6)	12
Grown Brown B 125 (Brn. 31)	10
Black GX 200% (Blk. 38)	10
Catechine 3G (Grn. 74)	4
Brown 3GN (Brn. 154)	4
Fast Brown B 125% (Brn. 31)	3
Fast Green BX 100% (Grn. 6)	3
Phenamine Black E-200 (Blk. 38)	2
Congo Red 4B (Red 28)	2
Brown M 100% (Brn. 2)	<1
Green WS 100% (Grn. 1)	<1
Diazo Black BH (Blue 2)	<1
Brown 3GN (Brn. 154)	<1
Allied Chemical	
Niagara Blue	<1
Erie Green GPD	<1

Source: Boeniger (1979)

Table 11. Residual Benzidine in Import Dye Samples

Dye name	Exporting country	Concentration of residual benzidine in ppm (w/w)
C.I. Direct Red 1	Belgium	224
C.I. Direct Orange 8	India	143
C.I. Direct Green 6	Holland	70
C.I. Direct Black 38	Egypt	53
C.I. Direct Black 38	Poland	40
C.I. Direct Black 38	Poland	38
C.I. Direct Blue 6	India	ID
C.I. Direct Black 38	India	9
C.I. Direct Blue 2	?	8
C.I. Direct Blue 2	Romania	8
C.I. Direct Blue 2	India	7
C.I. Direct Red 28	Romania	7
C.I. Direct Red 28	India	6
C.I. Direct Green 1	Poland	3
C.I. Direct Black 38	Holland	3
C.I. Direct Red 1	Poland	3
C.I. Direct Red 28	Poland	2
C.I. Direct Red 28	Belgium	2
C.I. Direct Blue 2	Poland	2
C.I. Direct Red 28	Korea	2
C.I. Direct Blue 2	Poland	1
C.I. Direct Red 37	Holland	1
C.I. Direct Brown/54	Poland	1
C.I. Direct Brown 1:A	Poland	1
C.I. Direct Red 28	Poland	<1
C.I. Direct Blue 2	Poland	0.4

Source: Boeniger (1979)

2. Laboratory Workers

Benzidine and its congeners are commonly used as analytical reagents in chemical, biochemical, and clinical laboratories, and the potential dangers of these compounds have been pointed out (Shriner et al. 1978). Levels of contamination and exposure probably vary widely; neither the size of the potentially exposed population nor the levels of actual exposure is known.

3. General Population

The general public is exposed mainly to finished dyes and pigments after they have been applied to textiles, leather, and other products; however, direct exposure to benzidine or its congeners, present as residual unreacted starting materials, or as breakdown products, is conceivable. The amounts of free benzidine in finished products, however, have not been reported (USCPSC 1977). The dyes and pigments in finished products are considered to be essentially "fast" (they do not migrate or wash out); major retailers' quality standards require colors to be durable through at

least 20 washings. One report on chemicals in wearing apparel, prepared for the U.S. Consumer Product Safety Commission (CPSC), stated that "there is little chance of dyes coming off in perspiration, saliva, or washings if labeling instructions are followed" (USCPSC 1977, p. III-14); however, no actual measurements of possible leaching were reported.

A report compiled by the Center for Occupational Hazards of the Artist-Craftsmen of New York, Inc., calls attention to the potential hazards to the general public--and artists and craftsmen in particular--presented by exposure to dyes and pigments specially packaged for craft use and the all-purpose dyes for general household use (Jenkins 1978). According to this report, direct dyes are the most common among those used in the crafts, and many of these dyes, as well as general purpose dyes, are thought to be derived from benzidine or its congeners. To investigate this question, Boeniger (1979) analyzed 15 consumer retail dyes purchased in arts and crafts shops in New York City. The dyes were chemically reduced and the relative amounts of aniline, benzidine, o-tolidine, and dianisidine were determined (Table 12). Nine of these dyes appear to be wholly or predominantly benzidine based and five o-tolidine based; only one of the 15 dyes contained no detectable benzidine or benzidine congener. Recent studies performed for the CPSC (1979), however, indicate that benzidine-based dyes are no longer being used for formulation of home dyes such as RIT. o-Tolidine- and dianisidine-based dyes are being substituted for the benzidine-based dyes. However, because the available inventory of benzidine-based dyes was not recalled and removed from the consumer market, such dyes could be available for several years.

Two other points raised by Jenkins (1978) are pertinent to an exposure analysis:

(a) The craftsman or home user is probably unaware of any potential hazard and does not use special precautions (as are expected to prevail in commercial dye plants) when using these dyes. Use of a cooking vessel with insufficient cleaning, for example, might lead to a significant amount of ingestion of dye.

(b) Some home or craft dyeing operations proceed in boiling water, at which temperature dyes based on benzidine substrates have been reported to decompose (Jenkins 1978). Presumably benzidine or other parent compounds are among the degradation products.

Table 12. Determination of Aniline, Benzidine, o-Tolidine, and Dianisidine in Retail Dyes

Company	Dye name	Reduced products (relative %)			
		Aniline	Benzidine	o-Tolidine	Dianisidine
FBS, Inc.	Fazan Brown #4029	1	99	0	0
"	Black #1628	2	98	0	0
"	Dark Blue #4025	0	0	0	0
Tintex	Black-Powder	2	98	0	0
"	Brown-Powder	1	99	0	0
"	Navy Blue #25	1	74	0	25
"	Royal Blue-Powder	0	1	98	1
"	Chocolate Brown-Powder	0	99	1	0
Aljo	Black-Cotton	12	88	0	0
"	Dark Brown	0	100	0	0
"	Imperial Blue	0	96	4	0
"	Royal Blue	0	2	96	2
RIT	Chestnut Brown #43	2	6	87	5
"	Navy Blue 30	2	0	93	5
"	Black 15	5	2	92	1

Source: Boeniger (1979)

VI. SUMMARY OF ISSUES INCLUDING VALIDATION AND INFORMATION NEEDS

A. Benzidine and Benzidine-Based Dyes

Direct exposures to benzidine, a human and animal carcinogen, probably occur primarily in occupational settings. Although direct exposure to benzidine during the manufacture of dyes and pigments appears to be controlled to a technologically feasible degree, several exposure problems result from the application of dyes contaminated with benzidine.

1. Workers may be exposed to as much as 500 ppm free benzidine in imported dyes; domestically produced dyes normally contain about 20 ppm free benzidine. Both the domestically produced and imported dyes are excluded from consideration in implementation of work practices prescribed by OSHA for dyes containing more than 0.1 percent (1,000 ppm) free benzidine. In addition, no occupational exposure limit has been established for either benzidine or benzidine-based dyes.

2. At least 85 percent of the dyes used are imported. Assuming that U.S. technology is not unique, one might question why these dyes have approximately twenty-five times the free benzidine that normally occurs in U.S. dyes. The lack of a system for tracking the flow of benzidine dyes in commerce, including imports, further complicates an assessment of the risks that may be associated with these dyes. For example, what are the individual dyes used for? Are exposures adequately controlled during use? Are some of the dyes used in uncontrolled circumstances, such as home craft uses?

Although one problem associated with the use of benzidine-based dyes is the level of free benzidine in the dyes, the exposure potential to benzidine may be greatly enhanced if one considers that a number of the benzidine-based dyes have been found to be metabolized to benzidine in mammalian systems. The following basic issues need to be considered:

(a) Are the metabolism studies on eight benzidine-based dyes and the demonstrated carcinogenicity of three dyes sufficient to make predictions about the dyes currently in commercial production or the larger number of dyes that can be synthesized by existing technology?

(b) Can all the dyes known to metabolize to benzidine be assumed to exhibit the same effects (i.e., carcinogenicity) as benzidine?

(c) Considering that the time-to-tumor interval is 5 weeks in rats orally dosed with benzidine-based dyes and somewhat longer in rats orally dosed with benzidine (the dose of benzidine was much lower than the dose of dyes) and that the pattern of tumors observed with the dyes is different from that observed in rats fed benzidine, can benzidine (or a benzidine metabolite) be

assumed to be the carcinogenic agent derived from the dyes? Can an adequate scientific basis be developed to explain differences in the time-to-tumor interval if metabolism of dyes to benzidine is used as the basis for a detailed risk assessment?

1. Validation Needs

Key review and validation needs for benzidine and benzidine-based dyes appear in the following areas:

(a) benzidine epidemiological studies;

(b) key animal carcinogenicity studies on benzidine and benzidine-based dyes (Direct Blue 6, Direct Black 38, and Direct Brown 95); and

(c) metabolism studies on the benzidine-based dyes.

B. o-Tolidine, Dianisidine, and Their Derivative Dyes and Pigments

Positive animal carcinogenicity studies have been reported for both o-tolidine and dianisidine; however, these studies may not be of sufficient strength to support a detailed risk assessment. The validity of the reported animal carcinogenicity studies, therefore, is a key issue in considering this subset of the category. Metabolic studies that clearly indicate that the derivative dyes and pigments are metabolized to the parent compounds are not available. One commercial o-tolidine-based dye (Trypan Blue) has been found to be carcinogenic, mutagenic, and teratogenic, although the carcinogenicity may be the result of components of the commercial product other than the primary o-tolidine-based dye. One o-tolidine-based pigment (Pigment Yellow 16) has been found negative in a carcinogenicity study and found not to be metabolized to o-tolidine by rats. Metabolism studies on several o-tolidine-based dyes (pigments) are currently in progress through the National Toxicology Program. The following questions should be addressed:

(a) Is there a need for additional carcinogenicity testing of o-tolidine and dianisidine?

(b) Is there enough structural similarity between benzidine- and o-tolidine- or dianisidine-based dyes to make basic conclusions about the risk associated with o-tolidine- and dianisidine-based dyes?

(c) Should the o-tolidine and dianisidine-based pigments be addressed separately from the dyes derived from these two benzidine congeners?

(d) Are the carcinogenicity, mutagenicity, and/or teratogenicity of Trypan Blue adequately characterized to permit a detailed risk assessment for that product?

C. Dichlorobenzidine and Its Derivative Pigments

1. Identified Issues

Dichlorobenzidine is reported to be an animal carcinogen, and at least one study in mice suggests that dichlorobenzidine may have some potential as a transplacental carcinogen. Reportedly this chemical also is photolyzed in the aquatic environment to benzidine and other products. Both benzidine and dichlorobenzidine have some potential for bioaccumulation. Exposure to and release of dichlorobenzidine into the environment currently are not controlled by Federal or state legislation. The three dichlorobenzidine-based pigments thus far tested have not been demonstrated to be carcinogenic; they have not been shown to metabolize to free dichlorobenzidine. The following questions may be asked with respect to these compounds:

(a) Does the weight of the accumulated evidence suggest that dichlorobenzidine is a carcinogen whose exposure and environmental release should be controlled?

(b) Are the existing metabolism and negative carcinogenicity studies sufficient to dispell concern that dichlorobenzidine-based pigments may be carcinogens?

(c) Is the hypothesis that structural features make dichlorobenzidine pigments resistant to enzymatic reduction (to dichlorobenzidine) sufficient to explain the lack of carcinogenicity observed with these pigments (also see General Issues [c]).

2. Validation Needs

The key review and validation needs for dichlorobenzidine and dichlorobenzidine-based pigments follow:

(a) A review of the animal carcinogenicity studies on dichlorobenzidine and Pigments Yellow 12, 13, and 83;

(b) Review and validation of the metabolism studies on dichlorobenzidine-based pigments;

(c) Review and validation of the study that suggests that dichlorobenzidine may be a transplacental carcinogen;

(d) Review and validation of the studies that show that dichlorobenzidine is photolyzed to benzidine in aqueous media; and

(e) Review and validation of bioaccumulation studies for both benzidine and dichlorobenzidine.

D. General Issues

One difficulty encountered in assessing the dyes and pigments available in the U.S. is obtaining current and specific production and importation information. The difficulty is created by the rapidly shifting

pattern of consumption. This is reflected in the differences between the list of dyes reported here as currently available and a list prepared by the Midwest Research Institute as a part of a materials balance (see Appendix A).

Because any future information-gathering studies will be seriously handicapped by these problems, the following questions must be addressed:

(a) Can the risk assessment be used for decision making on all dyes and pigments included in the category (i.e., all that can be synthesized by existing technology), or can conclusions be drawn for only a smaller subset of the category (i.e., those for which we have certain types of information)?

(b) Although this report addresses the primary benzidine congeners, other congeners (e.g., 3,3'-disulfo-, 2,2'-dichloro-, and 3-nitrobenzidine) exist. There is little, if any, available information on these congeners. Should these congeners be included in an assessment of the benzidine-based dyes and pigments or treated separately on an as-needed basis?

(c) Do factors such as the particulate nature of pigments, particle size, and possible differences between the transport and storage of particulate (pigment) and soluble (dye) materials suggest that dyes and pigments should be treated as separate issues?

1. Information Needs

Information needs, in part, will reflect the extent of the category and the thoroughness of additional assessment efforts. The information needed to evaluate the risk(s) associated with exposures to dyes and pigments that result from contact with textiles, for example, will differ from that required for determining the risk of worker exposures to dyes and pigments. Regardless of the decision concerning the type and extent of the assessment, the following information needs are anticipated:

(a) Level-III literature searches on specific topics;

(b) More in-depth materials balances for individual congeners and dyes and pigments (either individually or as a category);

(c) Information gathering under TSCA, section 8; and

(d) Monitoring studies to determine the quantity of dye or congener (e.g., dichlorobenzidine) that is released and distributed in the environment.

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APPENDIX A

Table A-1. Comparison of Dyes and Pigments Listed as "Currently Available in U.S."

Type of dye or pigment	Number of dyes or pigments listed		
	Listed in this report but not in USEPA (1979a)*	Listed in USEPA (1979a) but not in this report	Listed in this report and in USEPA (1979a)
Benzidine	6	--	16
o-Tolidine	15	1	7
Dianisidine	22	1	16
Dichlorobenzidine	--	4	5
Total	43	6	44

Source: USEPA (1979a)

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

This report assesses the risk to health and the environment presented by benzidine and three of its congeners (o-tolidine, dianisidine, and dichlorobenzidine) and by dyes and pigments derived from these compounds. Benzidine, o-tolidine, dianisidine and dichlorobenzidine are used almost entirely in the production of dyes and pigments used to color textiles, paper, leather, rubber, plastic products, printing inks, paints and lacquers.

Several potential risks have been identified through a preliminary analysis of the exposure and hazards associated with these compounds. These include: (1) the oncogenic risks to workers exposed to imported benzidine-based dyes that contain high concentrations of free benzidine; (2) a similar risk to workers using domestically produced benzidine-based dyes (because there are no occupational exposure standards for either benzidine or its derivative dyes); (3) the risk to the general population that may result from exposure to benzidine-based dyes in such products as textiles and home dyes; and (4) risks of toxicity to aquatic life that may result from release of dichlorobenzidine into the environment.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
benzidine; o-tolidine; dianisidine; dichlorobenzidine; bisazobiphenyl; dyes; pigments; carcinogens; mutagens; hazards; assessment	preliminary risk assessment; phase I assessment	

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